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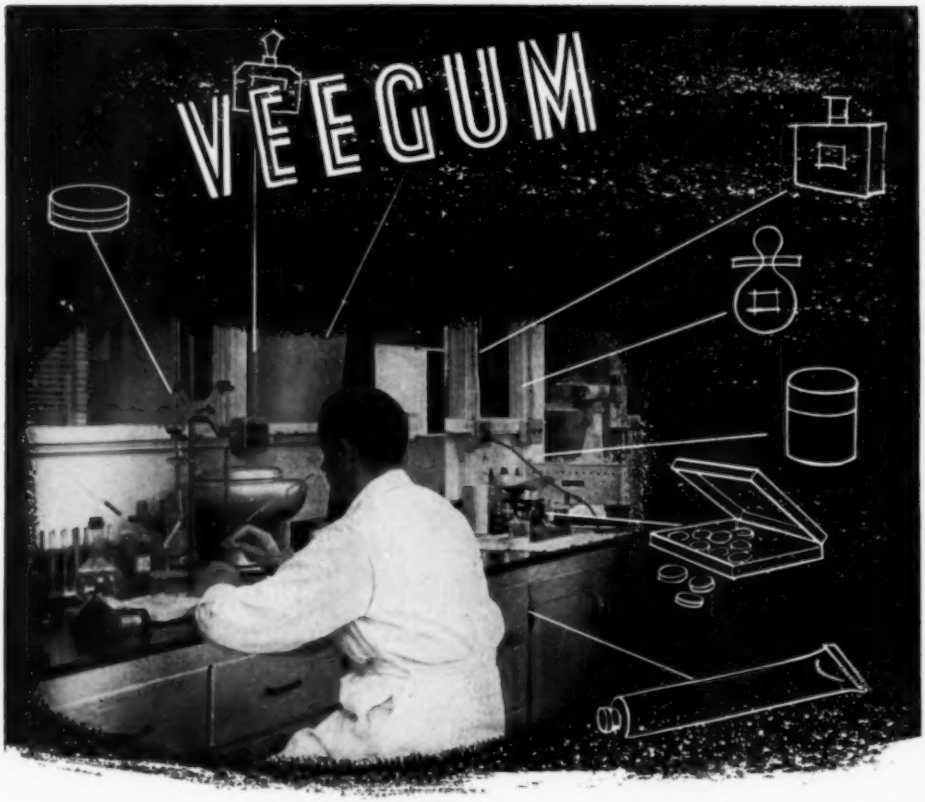

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Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1957 Chilean Iodine Educational Bureau, Inc., Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1957 Award, *nominations must be received on or before January 1, 1957.*

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The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

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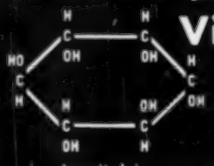

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLV — *NOVEMBER 1956* — NUMBER 11 — CONSECUTIVE NO. 22

The Determination of Free Menthol in Peppermint Oil by Chromatographic Analysis*

By S. K. HAMARNEH, M. I. BLAKE, and C. E. MILLER

A sensitive method has been developed for the determination of free menthol in peppermint oil. The menthol is separated by chromatographic adsorption and determined photometrically. Concentrations from 0.01 mg. to 0.13 mg. of menthol follow Beer's law.

THE OFFICIAL ASSAY for menthol in peppermint oil was developed sixty years ago by Power and Kleber (1). A variety of weaknesses and errors inherent in the method have been described in the literature over the years. Many suggestions and modifications have been recommended; but, in general, the method has remained the same. Several of these modifications are mentioned. Wilson (2) and Brignall (3) reviewed the difficulties of the assay in detail and suggested improvements. The saponification step, an important source of error, was eliminated. Wilson acetylated with acetic anhydride in pyridine, whereas Brignall used acetic anhydride in *n*-butyl ether. Christensen and Pennington (4) acetylated with acetyl chloride, while Jones and Fang (5) used acetic anhydride in pyridine. The menthol content was determined from the amount of acetylant consumed.

Nelson (6) stressed the importance of using analytically pure reagents and the highest purity anhydrous sodium acetate as the catalyst. Baldinger (7, 8) investigated the effect of varying the volume of alcoholic KOH solution and the effect of temperature changes in the saponification step. The time variable was studied in both the acetylation and saponification. According to Baldinger, a 1 per cent error is permitted by volatile oil companies in the assay of peppermint oil. Other modifications have been suggested by Fischer and Hall (9) and Elliot (10).

This report describes a method for determining the free menthol in peppermint oil. The menthol is separated from the oil by chromatographic adsorption on a silicic acid column. The menthol is eluted and the concentration determined photometrically. Advantage is taken of the red color which develops when menthol is treated with *p*-dimethylaminobenzaldehyde. Beer's law is followed over a usable concentration range. This color reaction was reported by Masamune (11) who sought a method for measuring free and conjugated menthol in blood and tissues. The color intensity of menthol samples was compared with the color of a phenol red standard in what was apparently a Duboscq-type colorimeter. The same color reagent is used in the official assay of ergonovine maleate injection

* Received April 13, 1956, from the School of Pharmacy, North Dakota Agricultural College, Fargo.
Presented to the Scientific Section, A. PH. A., Detroit meeting, April, 1956.

and tablets, ergotamine tartrate, ergotamine tartrate injection and tablets.

EXPERIMENTAL

Color Reagent.—The color-producing reagent was *p*-dimethylaminobenzaldehyde T. S., prepared as directed in the U. S. P. (12). A fresh solution of the reagent was made every four days.

Preparation of the Chromatographic Column.—The chromatographic tube employed in this study was made of Pyrex® glass with a fritted disk having a 10-mm. inside diameter and was 300 mm. in length. Preliminary studies indicated the following adsorbents to be unsatisfactory: alumina, calcium carbonate, charcoal, talc, and magnesium oxide. The solvents investigated in this work were acetone, benzene, carbon tetrachloride, chloroform, 1,4-dioxane, ethanol, ether, and petroleum ether. Chloroform was found to be the most useful. The adsorbent of choice was silicic acid, reagent grade, which was sifted through a 100-mesh sieve prior to use. A free flowing slurry was prepared in a beaker by stirring 9.5 Gm. of silicic acid with sufficient U. S. P. chloroform. The slurry was added slowly to the chromatographic tube, carefully avoiding the formation of air bubbles. The column was permitted to drain and was subsequently washed with 10 ml. of chloroform. The column prepared in this manner was 220 mm. in length and had an average flow rate of 0.1 ml. per minute. A slow flow rate proved more efficient for this work. A chloroform layer was maintained above the silicic acid until the column was ready for use.

Standard Curve for Menthol.—Stock solutions of U. S. P. menthol, racemic form, were prepared by weighing six samples of menthol ranging in weight from 6.0 mg. to 75.0 mg. Each sample was placed in a 25-ml. volumetric flask, flask A, and diluted to volume with chloroform. Further dilution was necessary in order that the menthol concentrations obey Beer's law. A volume of 1 ml. of each solution was transferred by pipet from flask A to a second 25-ml. volumetric flask, flask B, which was then diluted to the mark with chloroform. Exactly 1 ml. of each menthol solution from flask B was pipetted into a 50-ml. glass-stoppered cylinder. The prescribed volume of 5 ml. of color reagent was added from a buret, the cylinder was stoppered, shaken vigorously for one minute, and set aside for the reddish color to develop. It was observed that both length of time and menthol concentration influence color intensity. After one hour the colored solution was transferred to a colorimeter tube and readings were obtained with the Klett-Summerson photoelectric colorimeter using green filter No. 54 (500–570 mμ). An average of three readings was recorded for each of the six concentrations studied. The chloroform and color reagent, treated in the same manner as the menthol samples, served as the blank. Scale readings were directly proportional to the concentration of menthol and the absorbance of the colored solution. The readings were obtained at one-hour intervals after the initial reading. From these data the optimum time for color development was established. Figure 1 shows plots of scale readings of varying menthol concentrations at different time intervals. A two-hour time period

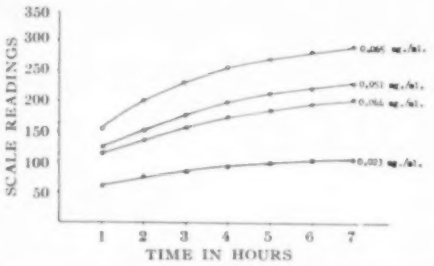

Fig. 1.—Effect of time on color reaction between menthol and reagent.

was selected as best suited for color production in this work. A standard curve, Fig. 2 was constructed for this time period showing scale readings and menthol concentrations (mg./ml.) in flask B. The standard curve was employed in subsequent work for determining menthol concentrations from scale readings. The curve was useful in the concentration range of 0.01 mg./ml. to 0.13 mg./ml. Dilutions in later work were designed so that the concentrations would fall in this range.

Chromatographic Analysis of Known Menthol Samples.—Prior to the analysis of peppermint oil a series of chromatographic analyses of pure menthol samples was carried out. Chloroform, U. S. P. grade, was used as the eluant for menthol. The column could be washed free of menthol rapidly and completely. Five solutions of known menthol concentration in chloroform were prepared in 25-ml. volumetric flasks. The concentrations were selected so that after proper dilution the readings observed with the colorimeter would fall on the standard curve. The solutions were treated in the same manner as those used to prepare the standard curve. This served to check the standard curve since the starting concentrations of menthol could be compared with the concentrations obtained from the curve.

The chromatographic column, ready for use, was permitted to drain. When the supernatant chloroform layer just disappeared on the column, a 1-ml. aliquot was pipetted from flask B (containing a 1-ml.

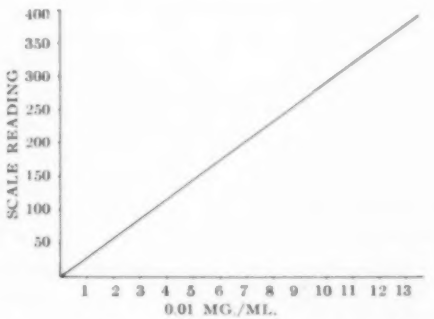

Fig. 2.—Standard curve for menthol in chloroform.

aliquot from flask A transferred to flask B and diluted to mark) to the column. Especial care was exercised in this manipulation so that no part of the column became dry. When the 1-ml. aliquot had just disappeared on the column, fresh chloroform eluant was added to the column and the eluate was collected in 10-ml. volumetric flasks. When the eluate in the first flask reached the mark, a second flask was placed beneath the column. This procedure was continued until six flasks were filled (a total of 60 ml. of eluate). Exactly 1 ml. from each flask was transferred to a 50-ml. glass-stoppered cylinder; 5 ml. of the color reagent was added from a buret and the cylinder was shaken vigorously for one minute. The contents of the cylinder was set aside for exactly two hours to permit the color to develop and then transferred to a colorimeter tube for subsequent measuring of the color. It was observed that the first 10 ml. of eluate developed no color. In most instances color was produced only in the second 10-ml. eluate, while in several determinations the second and third washings developed color with the second having a much greater color intensity. Subsequent washings remained colorless. It was concluded from this study that the menthol was washed from the column after 10 ml. and before 30 ml. of chloroform passed through the column.

The concentration of menthol washed from the column was determined by referring the scale readings to the standard curve. Where color developed in two flasks, the sum of both was calculated. The concentration of menthol obtained in this manner multiplied by ten gave the total menthol washed from the column. Multiplying this concentration by the dilution factor 625 gave the amount of menthol in the original sample. The following expression is applicable:

$$C \times 6{,}250 = \text{weight of menthol sample}$$

where C is the concentration of menthol in 1 ml. of the 10-ml. eluate fraction. The overall dilution factor is 6,250.

The data in Table I show a comparison of starting menthol weights (these weights agreed with the concentrations obtained from the standard curve when color was developed without chromatographing) with menthol recovered from the column.

TABLE I.—RECOVERY OF MENTHOL FROM KNOWN SAMPLES USING SILICIC ACID COLUMNS

Sample No.	Original Sample Weight, Gm.	Menthol Recovered, Gm.	Recovery, %
1	0.7500	0.7379	98.38
2	0.4375	0.4375	100.00
3	0.3813	0.3813	100.00
4	0.2500	0.2460	98.39
5	0.1875	0.1875	100.00

It should be noted that the weights of menthol selected for this study were about ten times those used in the preparation of the standard curve since only 1 ml. of the 10-ml. eluate fraction was used for color production.

Analysis of Peppermint Oil.—The peppermint oil employed in this work was U. S. P. grade, redistilled, obtained from Magnus, Mabee, and Reynard, Inc. Oil samples were treated in a manner similar to the known menthol samples studied in the preceding section. However, sample weights of the oil were approximately double those of the menthol samples, since peppermint oil, according to U. S. P. specifications, contains not less than 50 per cent total menthol. This assured that after proper dilution, the concentration would fall within the working portion of the standard curve. The data are shown in Table II. Oil sample weights are shown in the second column while the amount of menthol recovered from the oil by the method described in this paper is listed in the third column. The average yield of free menthol in the peppermint oil, as determined by this procedure, was found to be 50.46 per cent.

TABLE II.—DETERMINATION OF FREE MENTHOL IN PEPPERMINT OIL

Sample No.	Sample Weight, Gm.	Menthol Recovered, Gm.	Yield, %
1	1.3875	0.7000	50.45
2	1.3875	0.6979	50.30
3	1.3875	0.7075	50.99
4	1.2313	0.6188	50.26
5	0.6156	0.3125	50.76
6	0.1113	0.0556	50.00
7	0.1113	0.0562	50.49
		Average	50.46
		Av. Dev.	0.24
		Std. Dev.	0.30

Interference by Menthyl Acetate.—The major constituents usually found in peppermint oil were tested for possible color production with the color reagent used in this work. Only menthol and menthyl acetate gave a positive color reaction. Menthyl acetate constitutes not less than 5 per cent of peppermint oil according to U. S. P. specifications. Its concentration is an important factor in the official assay for total menthol. Because of the similarity in the chemical structures of menthol and menthyl acetate, the possible interference of the latter in the method presented here was investigated. Pure menthyl acetate was prepared according to the method of Miller (13). It gave a strong positive color reaction with *p*-dimethylaminobenzaldehyde T. S., similar in all respects to that produced by menthol. It is believed that the strongly acidic nature of the reagent hydrolyzes the ester with liberation of free menthol which is responsible for the color reaction. It is essential in the chromatographic isolation of menthol from peppermint oil that the relative adsorptive properties of the menthol and menthyl acetate vary sufficiently to permit a quantitative separation.

Pure menthyl acetate was chromatographed according to the procedure outlined for the known menthol samples described earlier. Menthol, it was noted, concentrates in the second 10-ml. fraction of the eluate. In the present study menthyl acetate did not appear in the first 60 ml. of the eluate. The fact that menthyl acetate is more difficult to remove from a silicic acid column than menthol by chloroform is evidence that menthyl acetate does not interfere with the free menthol determination.

SUMMARY AND CONCLUSIONS

1. The purpose of this project was to develop a more suitable method for the analysis of free menthol in peppermint oil. The need for such a method is emphasized in the literature.

2. The procedure described in this paper involves two important phases. The first step is the chromatographic separation of the menthol from the oil. After considerable investigation it was concluded that silicic acid serves as the best adsorbent and chloroform as the most suitable eluant. The oil is added to the column. The column is then washed with eluant. Complete removal of the menthol occurs in the 10- to 30-ml. eluate fraction. The menthyl acetate remains on the column. It does not interfere with the determination of free menthol.

3. The second phase is the photometric determination of menthol in the eluate. This is based on the color produced when menthol is treated with *p*-dimethylaminobenzaldehyde T. S. The color-producing procedure is a modification of the method reported by Masamune (11). The results (Table II) for the analysis of peppermint oil samples indicate that the method has merit.

4. Work is currently in progress in our laboratories applying this procedure to the analysis of menthol-containing pharmaceuticals and in the determination of menthyl acetate in peppermint oil. It might also be applicable to analysis of other volatile oils containing alcoholic constituents.

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The Relationship of Psyllium Seed and Various Fractions of the Seed to Kidney Pigmentation*

By F. COULSTON and J. C. SEED

Rats were fed various fractions of *P. ovata* and *P. arenaria* at a concentration of 10% in the diet for twenty-eight weeks. One of these fractions of *P. ovata* was Mucilose®, a commercially available product. Histologic pigmentation of the tissues was not observed, but the kidneys of the rats fed the fraction containing the tailings of *P. ovata* were slightly darker brown in color after six weeks of continuous feeding. Pigmentation is unlikely to occur in the kidneys of people taking Mucilose or similar psyllium preparations from which the black pigmented layer of the seed has been removed during pharmaceutical preparation.

PSYLLIUM is one of the older plant drugs which has maintained an important place in therapeutics in spite of the development of many newer synthetic compounds. It was described in Persia as early as the 10th century. Then under the popular name fleawort, Europeans began to use the Indian psyllium as a demulcent during the first part of the 19th century. A century later, use of the drug as a mild bulk laxative spread to the United States, where it soon attained a major place among laxative and cathartic medicaments.

In 1932, MacKay, Hall, and Smith (1) reported that the kidneys of rats became brown to black in color when 25% of the ground *Plantago psyllium* was added to the diet and fed over a period of 125 days. They ascribed this change in color of the kidney to a pigment material found in the cortex of the kidneys. The pigment was observed as fine brown granules in the epithelial cells of the collecting tubules, particularly in the proximal convoluted tubules and

* Received May 10, 1956, from the Section of Experimental Pathology and Toxicology, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

in Henle's loop. The nature of the pigment was not determined. Rats fed whole psyllium seed for 125 days and two dogs fed ground psyllium seed for 30 days did not have any changes in the kidney. Thienes and Hall (2) fed rats and cats diets containing 5% and 25% of *Plantago psyllium*, prepared as flakes in the proportion of three parts of psyllium to one part of agar. They also found macroscopically that the kidneys of the animals, fed the comminuted psyllium as an agar flake, became brown to black in color. However, only one of 20 rats on the 5% psyllium-agar diet and two of 20 rats on the 25% diet exhibited histologic pigmentation of the kidney. No deposits of pigment were observed in the kidneys of the cats although the kidneys appeared to be macroscopically slightly darker in color than were the normal kidneys. Urea-clearance studies were done on the rats and on nine human subjects. The clinical observations were made on people who had taken psyllium-agar flakes daily for 2 to 7 years. No evidence of kidney dysfunction was observed either in the rats or in the nine clinical cases studied.

Subsequently, the wide use of a selected portion of a special variety of psyllium seed, under the name Mucilose, made it of interest to determine whether the phenomenon of kidney pigmentation had any practical significance in relation to this product. The following study was done to determine whether other kinds of preparations of psyllium or botanical fractions of psyllium were implicated in the production of pigmentation in the kidneys of rats.

MATERIALS AND METHODS

White albino Sprague-Dawley rats, weighing approximately 240 Gm., were used in this study. The rats were divided into four groups of 30 rats each and into a fifth group of 45 rats. The rats were fed a ground, standard diet for 28 weeks composed of Purina pellets to which was added the substances shown in Table I, each in the proportion of 10% of the total diet.

Group 5, the control rats, was fed the standard

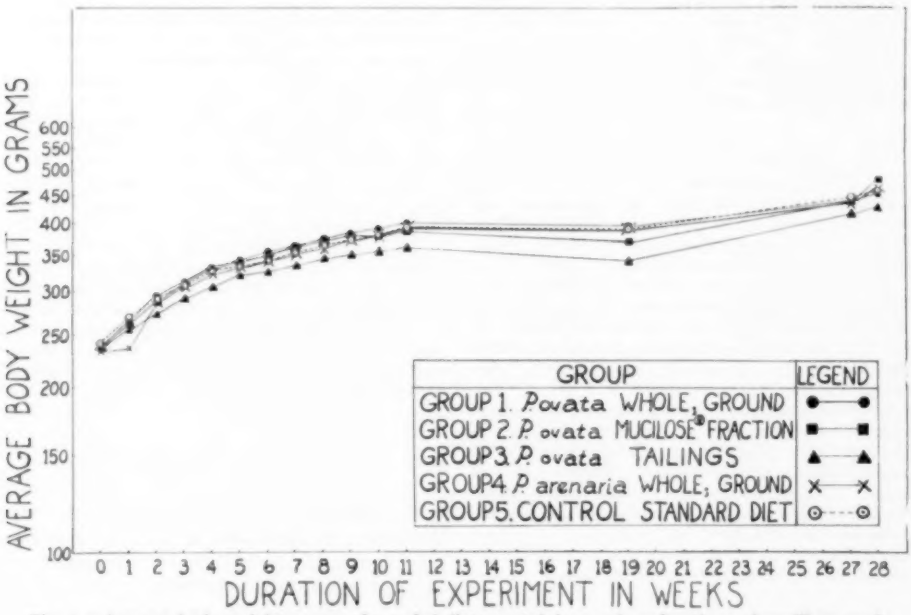

Fig. 1.—Average body weight curves of rats fed diets containing various fractions of psyllium seed.

TABLE I

	Group 1, *P. ovata*, Whole; Ground	Group 2, *P. ovata*, Mucilose Fraction	Group 3, *P. ovata*, Tailings	Group 4, *P. arenaria*, Whole; Ground
Remains, %				
On mesh 20	0.0	0.0	0.0	0.0
On mesh 50	45.0	49.0	61.0	68.0
On mesh 80	70.0	85.0	88.0	80.0
Passes through, %				
Mesh 80	30.0	15.0	12.0	20.0

diet of ground Purina pellets without the addition of any psyllium.

Mucilose is a commercial product (Winthrop Laboratories) prepared from the outer mucilaginous hull of *P. ovata*, commonly called blonde psyllium seed. The tailings are the remains of the hull after the mucilaginous substance is removed. *P. arenaria* was used instead of *P. psyllium* [used by MacKay, Hall, and Smith (1)] because the natural color of the former is darker than the latter. Furthermore, this species had not been studied previously by other investigators. The 10% level in the diet was selected on the basis of an estimated maximum human consumption of 0.33 Gm./Kg./day for Mucilose and of 1.0 Gm./Kg./day for whole psyllium seed. A 10% diet would provide the rats with a dose of Mucilose approximately 37.5 times the recommended human daily dose and 12.5 times the dose of psyllium seed per day.

The rats were examined and weighed weekly. Rats were sacrificed at four to six week intervals over the 28-week period of the experiment. The thoracic and abdominal viscera of each rat were observed macroscopically. Portions of the tissues were fixed in Zenker-formalin, embedded in paraffin, sectioned at 6 μ, and stained by the hematoxylin and eosin method. Frozen sections were prepared by the usual method and these were stained also with hematoxylin and eosin.

RESULTS

All of the groups of rats were normal in behavior and appearance throughout the experiment. As indicated in Fig. 1, the rats gained weight at a normal rate. A mild laxative effect was noticed only in the rats fed Mucilose, Group 2.

None of the kidneys from the rats fed the various fractions of psyllium were similar to the black kidneys described by MacKay, Hall, and Smith (1). However, the kidneys of the rats in Group 3, which were fed the tailings from *P. ovata*, became darker brown in color after six weeks of feeding. This color change persisted throughout the experiment without any variation in intensity. No macroscopic or microscopic lesion related to medication was observed in any of the rats. Pigment was not found histologically in any of the organs. Particular attention was given to the histologic preparations of kidneys, liver, and spleen.

DISCUSSION

Since MacKay, Hall, and Smith (1) described fine brown granules in the epithelial cells of the tubules of the rat, an explanation of the negative results reported here should be sought. Thienes and Hall (2) were also unable to find pigmentation in the tubule epithelial cells of the kidneys of the cat, but did find pigmentation in two rats fed a 25% diet of psyllium and in one rat on a 5% psyllium diet. This indicated a low level of pigmentation as compared to that found by MacKay, Hall, and Smith (1) and is more in accord with the data presented here. A direct comparison to the work of MacKay, Hall, and Smith (1) is difficult since those authors did not state how many rats were used in their studies.

The fact that *P. psyllium* was not used in our studies must be emphasized. As pointed out by Youngken (3, 4), the classification of psyllium seed was not clear at that time. The possibility exists that MacKay, Hall, and Smith (1) and Thienes and Hall (2) used a mixture of psyllium seeds or even a different species than that indicated in their work. Furthermore, MacKay, Hall, and Smith (1) used a 25% diet of psyllium whereas in our experiments a 10% diet was employed. The 10% diet is a more realistic figure as compared to the human dosage. MacKay, Hall, and Smith (1) indicated that rats fed for less than 125 days on the 25% diet did not develop pigmentation in the kidney tubules.

The darkening of the kidneys produced in the rats in Group 3, fed tailings from *P. ovata*, indicated that the pigment-producing fraction may be in the cellulose layer of the hull of the seed. Ground *P. arenaria* is a very dark brown powder whereas the ground tailings of *P. ovata* are tan. Therefore, it was surprising to find that the macroscopic pigmentation of the kidney occurred only in the rats fed the tailings of *P. ovata*. This could be explained by differences in absorption or metabolism of the pigment in the two substances. Physical size differences of the particles of psyllium can be disregarded as is apparent from the sieve analysis. Obviously, there was no relationship between the color of the psyllium seed and the production of pigmentation. Since Mucilose is relatively free of the botanical fraction related to pigment formation, no pigmentation should occur following long, continued use of Mucilose.

Worthy of mention is the fact that Thienes and Hall (2) studied nine human subjects who had taken relatively large amounts of psyllium for two to seven years without finding any abnormality in kidney function or in the composition of the urine. When one considers the many necropsies performed on patients using psyllium and the lack of reports of kidney pigmentation, this phenomenon either does not occur in human subjects or, if it does, the pigmentation is so minimal that it is not reported by the pathologist.

CONCLUSIONS

1. Rats fed Mucilose and three other preparations of psyllium seed at a 10% concentration in the diet for 28 weeks were normal in appearance and behavior throughout the experiment. The body weight gain of the rats was normal.

2. No pigmentation of the epithelial cells of the collecting tubules of the kidneys was observed. No macroscopic or microscopic lesion attributable to medication was observed in any of the rats. The kidneys of the group of rats fed tailings from *P. ovata* were slightly darker brown than normal after six weeks of continuous feeding. The intensity of color did not vary from this point until the end of the experiment. When pigmentation does occur, it is probably related to this botanical fraction of the hull of the seed.

3. These data are not in accord with the report of MacKay, Hall, and Smith (1) but are more like the data reported by Thienes and Hall (2).

4. These observations clearly indicate that pigmentation of the kidneys is unlikely to occur in patients given Mucilose or similar psyllium preparations in which the black pigmented layer has been eliminated from the seed during the pharmaceutical processing.

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The Isolation of N-Methyl beta-Phenylethylamine from *Acacia berlandieri**

By BENNIE J. CAMP and CARL M. LYMAN

The isolation and characterization of a toxic principle from *Acacia berlandieri* is described. The compound was identified as N-methyl β-phenylethylamine.

THE CONDITION known as "Limberleg" or "Guajillo Wobbles" occurs in range sheep and goats during and immediately following prolonged periods of drought in the part of West Texas where the leguminous shrub, *Acacia berlandieri*, commonly called guajillo, is the dominant and often the only vegetation available for grazing livestock.

Price and Hardy (1), by means of animal feeding experiments, have shown conclusively that limberleg in these animals is due to the guajillo plant. Fraps (2) found that both the leaves and the fruit of the plant gave a positive test for alkaloids.

The loss from limberleg during the periods of prolonged drought amounts to thousands of sheep and goats, and mortalities on some of the more severely affected ranches may reach 65% of the mature animals. It is a conservative estimate that 1,500,000 sheep and 250,000 Angora goats graze in the part of West Texas where *Acacia berlandieri* is the dominant vegetation.

The purpose of this paper is to report the isolation of N-methyl β-phenylethylamine from *Acacia berlandieri*.

Barger and Dale (3) have described the relative pressor effects of β-phenylethylamine and its N-methyl and hydroxy derivatives. Hjort (4) has described some of the physiological properties and relative toxicities of certain N-methylated β-phenylethylamines.

EXPERIMENTAL

Isolation.—The air-dried leaves from the guajillo plant were extracted repeatedly with a 3% solution of hydrochloric acid until the acid extract failed to give a positive test for alkaloids with Valser's reagent.

The extract was then adjusted to *p*H 11 with concentrated potassium hydroxide, and the alkaloid was separated from the extract, containing plant pigments and fatty acids, by means of steam distillation. The alkaloid was adsorbed on an ion exchange column by passing the steam distillate through a column of IRC-50 (H) at the rate of 1 ml. per 10 seconds. The base was then eluted from the column with a 5% solution of hydrochloric acid. The acid eluate was then adjusted to *p*H 11 with concentrated potassium hydroxide, and the free base was extracted with chloroform in a separatory funnel.

The chloroform extract was concentrated by means of open evaporation at room temperature. The oily base was converted to the hydrochloride salt by passing anhydrous hydrogen chloride into an ether solution of the compound.

By means of paper chromatography, it was possible to demonstrate the presence of only one alkaloid in the oily base obtained by this isolation procedure. The material was chromatographed on Whatman No. 1 in a solvent phase consisting of *n*-butanol:acetic acid:water = 100:4: saturated with water.

The alkaloid was detected by spraying the paper with Dragendorff's reagent, which formed a red spot on a pale orange background having an R_f value of 0.65. Using this technique, it was also possible to show the presence of only one alkaloid in crude steam distillates.

Preparation of Resin Column.—The resin column (4.6 cm. i. d. x 120 cm.) was prepared by slurring approximately 250 Gm. of washed Amberlite®

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The authors wish to acknowledge the assistance of Horace Fawcett and Dr. William T. Hardy in the collection of plant material for this investigation.

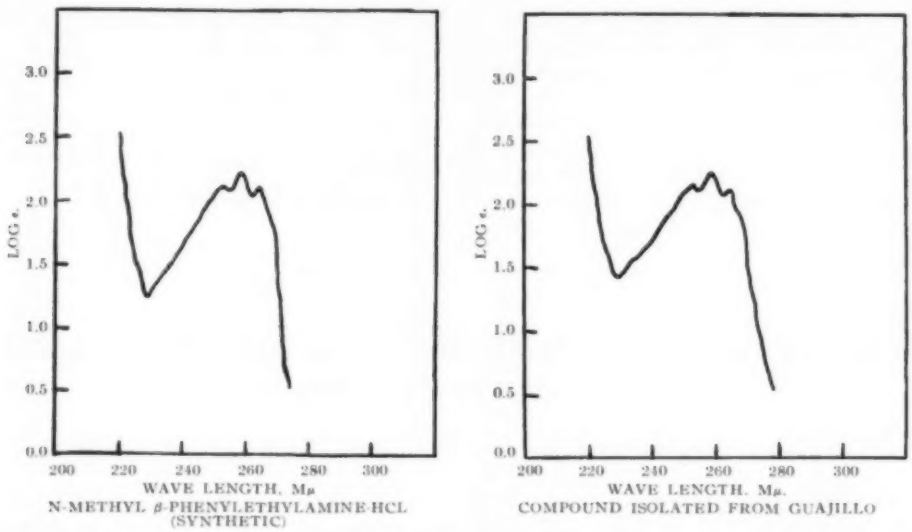

Fig. 1.—Comparison of ultraviolet absorption spectra of the hydrochloride salts of N-methyl β-phenylethylamine and isolated compound.

IRC-50 (H) into the exchanger tube. The bed was backwashed with tap water for about five minutes at such a rate that it expanded to about 1½ times its drained volume, permitting the column to form by sedimentation. The resin was regenerated by passing a 5% solution of hydrochloric acid through the bed until the *p*H of the effluent was 1 or less. The resin column was then washed with distilled water until the effluent reached a *p*H of 5.5–6.0.

Toxicity.—The toxicity of the compound was ascertained by the intraperitoneal injection of the hydrochloride salt (25–100 mg./ml.) into young rats. The physiological symptoms manifested in the experimental animals were similar in most respects, but the time required to cause death varied with the amount of the material injected. In a typical experiment, rats weighing approximately 75, 80, 140, and 150 Gm. received 50, 100, 42.5, and 42.5 mg. of the salt, and death occurred in twelve, seven, seventeen, and sixteen minutes after the injection, respectively. The symptomatological effects observed were: progressive dyspnea, clonic convulsions, ophthalmic dilatation, incoordination of posterior limbs, and cyanosis. Post-mortem examination revealed only congestion of abdominal viscera.

Analytical.—Quantitative analysis of the elements and molecular weight determinations postulate the following molecular formula for the alkaloid isolated from *Acacia berlandieri:* $C_9H_{12}N$.

Anal.—Calcd. for $C_9H_{12}N$·HCl: C, 62.96; H, 8.22; N, 8.15; Cl, 20.65. Found:[1] C, 62.13; H, 8.10; N, 7.83; Cl, 20.76.

[1] The microchemical analyses were carried out by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Micro-Tech Laboratories, Skokie, Ill.

The preparation of a series of derivatives which were suitable for the identification of the compound as N-methyl β-phenylethylamine are presented. All melting points were carried out on a Fisher-Johns melting apparatus, and are uncorrected. Nitrogen was determined by a micro Kjeldahl method.

Preparation of Hydrochloride.—The acid salt was prepared by passing anhydrous hydrogen chloride into an ether solution of the liquid base. The salt crystallized from the ether solution as white plates. The compound was purified by precipitation from absolute alcohol by the addition of anhydrous ether. The compound was found to have different melting points from different preparations, m. p. 157–158°; reported m. p. 157–158° (5); m. p. 161–162°; reported m. p. 161–162° (6); m. p. 164–165°; reported m. p. 164–164.9° (7). In each instance, it melted to a viscous oil with no effervescence.

Anal.—Calcd. for $C_9H_{12}N$·HCl: N, 8.15. Found: N, 7.85.

Preparation of Picrate.—This salt was obtained when a saturated, aqueous solution of picric acid was added to an aqueous solution of the hydrochloride. The picrate was purified from hot water. The crystals melted to a clear oil with no effervescence, m. p. 140–141°; reported m. p. 141° (8), and 141–142° (6).

Preparation of Oxalate.—The oxalate was prepared by adding the base to a cold, saturated, aqueous solution of oxalic acid. The oxalate was purified from hot water, which melted to an oil with effervescence, m. p. 187–188°, reported m. p. 183–184° (8).

Anal.—Calcd. for $C_{11}H_{13}O_4N$: N, 6.22. Found N, 6.13.

Preparation of Phenylthiourea.—Equal amounts

of the amine and phenyl isothiocyanate were mixed in a test tube and shaken. The material solidified after being placed in an ice bath. The powdered material was washed with 50% ethanol, and recrystallized from 95% ethanol. The crystals melted to an oil, m. p. 110–112°; reported m. p. 113–114° (8).

Anal.—Calcd. for $C_{16}H_{18}N_2S$: N, 10.37. Found: N, 10.94.

Preparation of Mercuric Chloride Derivative.—A 10% solution of mercuric chloride was added to an aqueous solution of the amine hydrochloride. The salt melted to a clear oil with no effervescence, m. p. 174–176°; reported m. p. 172–173° (8).

Preparation of Chloroplatinate.—Hydrochloroplatinic acid (5 Gm./100 ml. water) was added to an aqueous solution of the hydrochloride. It was recrystallized from hot water, which melted with effervescence, m. p. 201–202°; reported m. p. 212° (8), and 220–221° (6).

Preparation of Picrolonate.—This salt was prepared by adding a saturated alcohol solution of picrolonic acid to an aqueous solution of the hydrochloride. The crystals melted to a red oil with slight effervescence, m. p. 218–222°; reported m. p. 217–218° (8).

N-Methyl β-Phenylethylamine.—The oily base isolated from the plant material was purified by *in vacuo* distillation. It boils at 67.40 (corrected) at 3.25 mm. pressure; reported b. p. 112.5–115° at 36–40 mm. pressure (8). It is a strong base which absorbs carbon dioxide from the atmosphere. It has a distinct amine odor, and shows a strong alkaline reaction with litmus paper.

Ultraviolet Absorption Spectra.—To further substantiate the identity of the alkaloid as N-methyl β-phenylethylamine, the ultraviolet spectra of the hydrochloride salts of the isolated compound and N-methyl β-phenylethylamine were determined. The amine was synthesized according to the method of Johnson and Guest (8). The acid salt of the synthetic compound melted at 164–165°. Ultraviolet measurements were made using a Beckman-DU spectrophotometer in 95% ethanol. The ultraviolet absorption curves obtained are compared in Fig. 1.

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Observations on *Rauwolfia cambodiana**

By HEBER W. YOUNGKEN†

DURING THE PAST YEAR the roots of *Rauwolfia cambodiana* Pierre ex Pitard (1), a shrub of the *Apocynaceae* attaining a height of 1 m. and bearing opposite to 3-whorled oblong to oblanceolate, petiolate leaves, subterminal umbellate cymes of small, pink to white, tubular flowers and compressed ovate drupes, have been offered to prospective purchasers in the United States as a substitute for *Rauwolfia serpentina* root and possible source of reserpine. The plant (Fig. 1) is native to Indo-China and also grows in Thailand where it is known as "Rayom."

Samples of two lots of these commercial roots purported to have been shipped from Thailand were examined by the writer, organoleptically and microscopically, according to standard pharmacognostic methods similar to those reported in his earlier papers on roots of other *Rauwolfia* species (2–4). Verification of the

Fig. 1.—*Rauwolfia cambodiana* Pierre ex Pitard. Leaf and flowering branches. ×.

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botanical identity of these samples was made by comparison with herbarium material supplied by the Section of Systematic Botany, Department of Agriculture and Fisheries of Siam.

The purpose of this paper is to give a report of the results of a preliminary study of the root of *Rauwolfia cambodiana* and to point out the differences noted to date between it and the root of *Rauwolfia serpentina*.

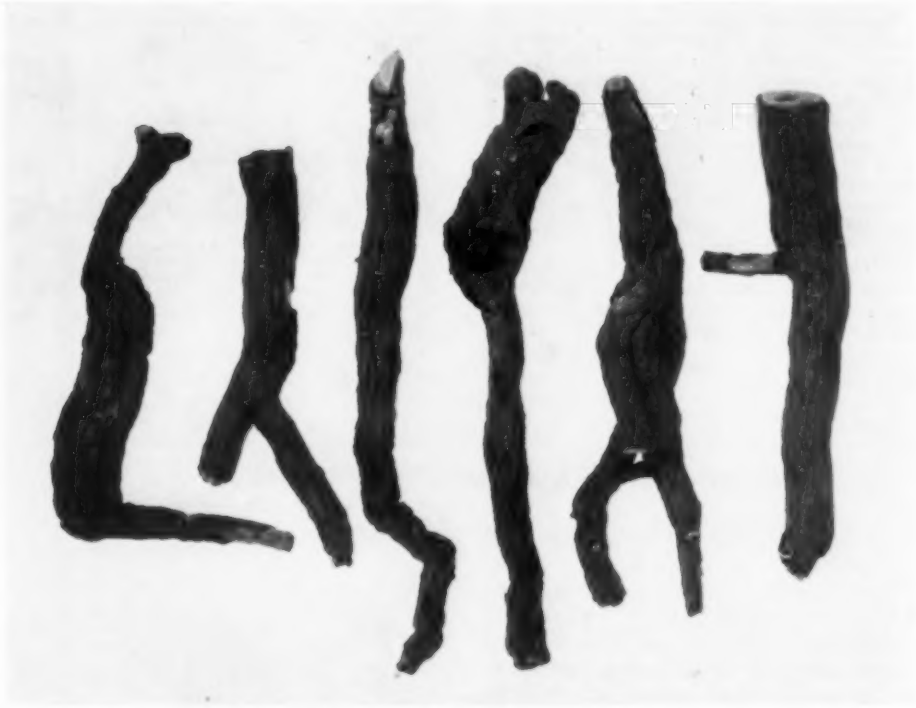

Fig. 2.—Root segments of *Rauwolfia cambodiana*. × 1/2.

EXPERIMENTAL

Physical Characteristics.—The roots studied ranged from 2 mm. at the tip to 2.5 cm. in diameter at the crown. They were elongate-conical, the segments up to 16 cm. in length, simple or 2- to 3-branched, straight, curved, or tortuous (Fig. 2). Externally, while occasionally longitudinally wrinkled in thin pieces, most of the segments exhibited many irregular longitudinal ridges or cork wings and furrows. In thick segments of these roots as many as 14 of these ridges occurred in the root circumference. The cut transverse surface of a segment measuring 13 mm. in diameter showed the ridge part of the bark to have a diameter of 6 mm. and the wood portion a diameter of 7 mm. Some of the segments bore rootlet stumps. The external color ranged from yellowish brown to buff to orange-brown, where abraded. The fracture is short, some pieces exhibiting projecting slivers of cork along the margin. The color of the fractured surface was brown in the bark region and pale yellow in the wood. The odor was indistinct; the taste bitter.

Upon boiling in water, a pinkish color developed in the wood. A solution of 1 part of conc. nitric acid and 2 parts of distilled water, when applied to the cut transverse surface of the root, colored the bark and wood pink, followed by purple, changing to purplish brown and brown. In *Rauwolfia serpentina* root, the same test gives a yellow to orange color to the wood, changing to pink and purple, and a purplish brown to the bark.

Histology.—Transverse sections of *R. cambodiana* root exhibited a stratified cork consisting of alternating tangential zones of larger and smaller cork cells both with thin lignified walls (Fig. 3). The larger cork cells occurred in the broader zones and were arranged in radial rows. They measured up to 60μ in radial length and up to 48μ in tangential width. The smaller cork cell zones occurred in wavy irregular crenate bands of collapsed or compressed cells, 2 or 3 layers in depth. They were up to about 47μ in length and up to 20μ in width. The phelloderm consisted of a narrow zone of tangentially elongated starch-bearing parenchyma. The phloem showed a broad region of starch-bearing phloem parenchyma and sieve tissue traversed by

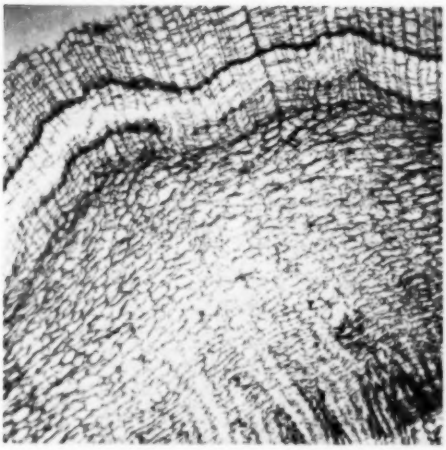

Fig. 3.—*Rauwolfia cambodiana*. Transverse section of a representative portion of the bark of the root 10 mm. in diameter. ×30.

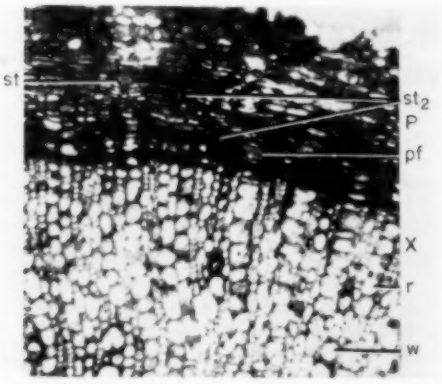

Fig. 4.—Transverse section of a portion of another root of *Rauwolfia cambodiana*, 8 mm. in diameter, showing phloem (*P*) with an isolated stone cell (*st*), groups of thick-walled stone cells (st_2), and a group of nonlignified phloem fibers (*pf*); outer region of secondary xylem (*X*) whose rays (*r*) and xylem wedges (*w*) are curves; vessel. Photomicrograph, ×42.

phloem rays. This region varied in different roots in respect to the presence of phloem sclerenchyma. In some roots phloem sclerenchyma only occurred in segments 8 mm. or more in diameter (Fig. 4). In others it occurred in sections as narrow as 2 mm. in diameter.

The phloem sclerenchyma consists of sclereids and fiber-like stone cells which were isolated and in small groups mostly of 2 or 3. The sclereids are laminated and up to 133μ in diameter with irregular lumina and lignified walls. The fiber-like stone cells are more or less rod-like and measured up to 175μ in length. The phloem rays were mostly up to 1 to 5 cells in width. Groups of fibers with nonlignified walls were also noted in the secondary phloem (Fig. 6, *pf*).

The cambium consisted of a wavy line of thin-walled meristematic cells and was broad in many sections.

The secondary xylem (Fig. 5) was relatively broad and composed of numerous, usually curved, wood wedges separated by sinuous xylem rays. Many conspicuous annular rings of small celled elements with thick walls occurred in the xylem of thicker segments. The wood wedges consisted of wood fibers, wood parenchyma, pitted and border-pitted vessels and tracheids. Pitted and border-pitted vessels are up to 437μ in length and usually up to 43μ in width, occasionally up to 57μ in width. They possessed oblique to transverse perforation plates on their end walls or, in numerous instances, openings on their lateral walls near the end of the

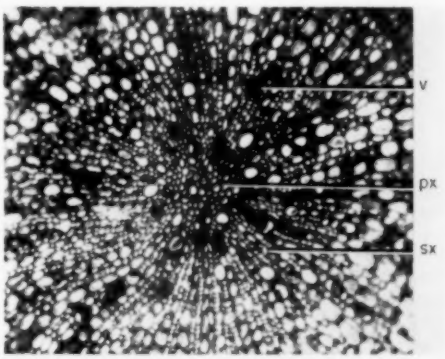

Fig. 5.—Central region of the same section of the root of *Rauwolfia cambodiana* illustrated in Fig. 4, showing the inner region of the secondary xylem (*sx*) and primary xylem (*px*). The dark objects occluding the lumina of some of the vessels (*v*) represent deposits of gummy lignin. Photomicrograph, ×30.

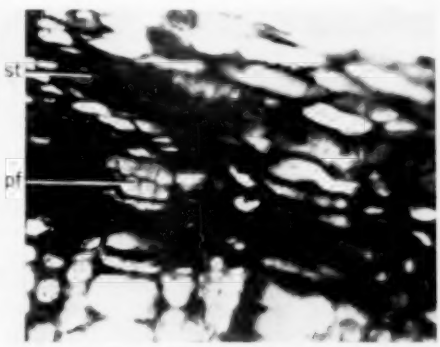

Fig. 6.—Transverse section of the inner region of the secondary phloem, cambium and outer layers of the secondary xylem of *Rauwolfia cambodiana* root representing an enlarged small area of the same section illustrated as Fig. 4, and showing an elongated group of thick-walled stone cells (*st*), and a group of phloem fibers (*pf*) with thick, nonlignified walls. Photomicrograph, ×270.

vessel elements. Their walls were lignified. The wood fibers were mostly sinuate with pointed, often curved ends, many showing serrations above the ends. Their walls were lignified and possessed oblique pits. They were up to 1650μ in length. The wood parenchyma cells possessed pitted lignified walls and contained starch. The xylem rays were sinuate and from 1 to 4, occasionally 1 to 5 cells in width. Many were 1 to 2 cells in width. Their cells possessed lignified, pitted walls. The primary xylem is tetrarch (Fig. 5).

The starch grains were single and from 2- to occasionally 3-compound, the individual grains spheroidal, ovate to truncate and plano- to concavo-convex, the hilum centric to eccentric, often several to many cleft. The grains were mostly up to 20μ in diameter, occasionally up to 30μ in diameter. Most of the starch occurred in the xylem.

In longitudinal sections the cork cells were polygonal to irregularly polygonal and up to 90 × 48μ.

Rauwolfia cambodiana root differs from that of *R. serpentina* in developing thick cork ridges or wings of considerable depth, in the cork cell walls being thinner, and in the cell walls of the narrower bands of cork being lignified, in possessing sclerenchyma elements in the phloem, in the curved character of its wood wedges and wood rays, in the development of longer wood fibers, in the smaller maximum size of the starch grains, and in the narrower maximum width of its xylem rays.

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The Effect of Selected Phenols on the Formation of Hydrogen Peroxide by Calamine Liniment N. F. IX*

By JOHN C. YOUNG and EARL P. GUTH†

When phenol, resorcinol, resorcinol monoacetate, betanaphthol, and pyrogallol were added to calamine liniment and the product irradiated with ultraviolet light, a variation in the amount of hydrogen peroxide produced was noted. The amount of hydrogen peroxide produced varies with the particular phenol, the concentration of phenol, and the age of the product.

ZINC OXIDE is one of the most frequently used chemicals in medicinal products intended for use in the treatment of dermatological conditions. It is official in the Pharmacopeia of the United States, XIV (1), as zinc oxide and in preparations such as calamine, calamine lotion, zinc oxide ointment, and zinc oxide paste. In the National Formulary IX (2), it is found in the form of prepared neocalamine, neocalamine lotion, neocalamine liniment, neocalamine ointment, phenolated neocalamine lotion, calamine liniment, calamine ointment, phenolated calamine lotion, zinc oxide hard paste, zinc oxide soft paste, and zinc oxide paste with salicylic acid. Zinc oxide is often compounded with other materials, such as phenols, organic acids, hydrocarbons, water, and glycols, etc.

Reese, Mathias, Minardi, Blubaugh, and Guth (3–6) have studied the "Formation of Hydrogen Peroxide in Calamine Lotions," "The Effect of Various Phenols on the Formation of Hydrogen Peroxide by Zinc Oxide," "The Effect of Time on the Hydrogen Peroxide Production by Calamine Lotions," "The Effects of Lipids Upon the Quantity of Peroxides Produced by Photosensitized Zinc Oxide," respectively. The first three studies were devoted to suspensions of zinc oxide in aqueous preparations. The fourth study was concerned with emulsion-type preparations containing zinc oxide. The emulsions were primarily of the water in oil (w/o) type.

Blubaugh (6) found that the addition of fixed oils markedly reduced the quantity of peroxides produced by photosensitized zinc oxide and that there was a wide variation among the several fixed oils used. It was also shown that the addition of calcium hydroxide solution to the oil-zinc oxide mixture increased the quantity of peroxides with some oils and decreased the amount produced with other oils.

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The photochemical formation of hydrogen peroxide in aqueous solution under the influence of biological sensitizers is assumed to proceed as follows: $2H_2O \rightarrow H_2O_2 + 2H(I)$; $2H + O_2 \rightarrow H_2O_2(II)$; $A + H_2O + O_2 \rightarrow AO + H_2O_2(III)$. "A" is the organic stabilizer. Although light energy is sufficient to promote these reactions, the heat produced by (II) and (III) would accelerate reaction (I) (7).

The presence of a small concentration of one of the many water-soluble easily oxidized compounds (termed "stabilizers or additives") in the zinc oxide, water, and oxygen mixture increases the rate of peroxide formation greatly. In such a system, zinc oxide is a photosensitizer for hydrogen peroxide formation. Hydrogen peroxide is formed primarily in the region of light absorption by zinc oxide, 2.500 A. There is no apparent net change by zinc oxide. In general, the light-activated zinc oxide catalyzes the occurrence of a normal exothermic reaction (8).

EXPERIMENTAL

Preparation of Samples

The samples were prepared in amounts of 150 ml. each. Twenty-four grams of a zinc oxide-calamine mixture (previously mixed and containing 0.25 per cent Fe_2O_3) was weighed in a tared eight-ounce presciption square; 68.62 Gm. of olive oil was then added and the mixture shaken for twenty minutes on a mechanical shaker. After this mixing, 68.45 Gm. of calcium hydroxide solution was added to the containers. The containers were closed immediately. This maintained the strength of the calcium hydroxide solution. The containers were returned to the mechanical shaker for an additional sixty minutes.

When phenols were added to the liniment the correct percentage was dissolved or suspended in the olive oil just before the oil was added.

When the mixture consisted of two ingredients, i. e., calamine-zinc oxide mixture and oil, calamine-zinc oxide mixture and calcium hydroxide solution, or oil and calcium hydroxide solution, the same procedure of mixing and shaking was employed.

Five-gram samples of the calamine liniments were used for irradiation. When only one or two of the components were studied, only that amount of the ingredient represented in a 5-Gm. sample of calamine liniment was irradiated. These amounts were determined on a weight basis. A 5-Gm. sample was made up of 0.750 Gm. of zinc oxide, 2.130 Gm. of olive oil, and 2.125 Gm. of calcium hydroxide solution.

Method of Irradiation

Fifty milliliters of distilled water was placed in an 800-ml. Pyrex beaker. Then exactly 5 Gm. of a calamine liniment was added to the distilled water. After the sample was added an additional 50 ml. of distilled water was added to the beaker.

When only one or two of the ingredients were to be irradiated, the samples consisted only of the amount of those ingredients present in a 5-Gm. sample.

Four beakers of each sample were prepared and then placed in the water bath under the ultraviolet lamp. The water bath was cooled by running water. Glass stirrers driven by a Cenco Stirrer Variable Speed Motor at 530 r. p. m. were utilized as a means of agitation of the samples during irradiation. The equipment was arranged so that each of the beakers was the same distance from the ultraviolet lamp. The distance from the lamp to the surface of the liquid was 35 cm.

The irradiation unit was the same one designed and used by Blubaugh (6). The unit was completely enclosed within aluminum sheeting. The beakers rested on a false aluminum bottom in the water bath and were held in place by a sheet of aluminum containing holes that were the same diameter as the 800-ml. beakers. The level of the water in the bath was kept at a constant level slightly above the contents of the beakers.

Irradiation time for all samples was thirty minutes.

Method of Assay

The iodometric method of Kolthoff (9) as modified by Chari and Qureshi (10) was utilized for the determination of peroxides.

The irradiated samples were filtered immediately through Whatman No. 1 filter paper, giving a clear filtrate. The first 20 ml. of the filtrate was discarded. To a 50-ml. portion of the clear filtrate in an iodine flask, the following reagents were added in the order given: 10 ml. 2 *M* sulfuric acid; 10 ml. 0.2 *N* potassium iodide; 5 ml. 0.04 *N* ammonium molybdate. The flasks were well shaken and placed in the dark for ten minutes. The liberated iodine was titrated with 0.01 *N* sodium thiosulfate, using freshly prepared starch test solution as the indicator.

Irradiation of Samples

Hydrogen Peroxide Formation by Calamine Liniment and the Various Ingredients of This Liniment.—The initial portion of the experimental work consisted of determining the amount of hydrogen peroxide formed by the ingredients of calamine liniment singly, then all the possible combinations, and lastly, calamine liniment.

The Effect of Various Phenols on the Formation of Hydrogen Peroxide by Calamine Liniment.—The principal portion of the experimental work consisted of determining the effect of various phenols on the formation of hydrogen peroxide by calamine liniment.

DISCUSSION

Irradiation of the basic ingredients by ultraviolet light produced a wide variance in the quantity of hydrogen peroxide. The results follow the pattern previously demonstrated by Blubaugh and Guth (6) (Table I). The results show that the total peroxide production by the irradiation of zinc oxide and water with ultraviolet light is greatly reduced by the presence of other ingredients of calamine liniment.

Zinc oxide (0.75 Gm.) the amount present in

5.0 Gm. of calamine liniment produced 865 μg. of peroxide while the zinc oxide-calamine mixture which contains ferric oxide produced 119 μg. The incorporation of olive oil reduced the peroxide production to 121 μg. Other comparisons are shown in Table I. The liniment produced 172 μg. of peroxide.

TABLE I.—MICROGRAMS OF PEROXIDES FORMED BY INGREDIENTS OF CALAMINE LINIMENT N. F. IX

Irradiated Material	μg.
Distilled water, 100 ml.	67
Olive oil, 2.130 Gm.	114
Calamine-zinc oxide mixture Fe_2O_3 0.25%, 0.750 Gm.	199
Olive oil, 2.130 Gm.; calamine-zinc oxide mixture, 0.750 Gm.	121
Calamine-zinc oxide mixture, 0.750 Gm.; lime water, 2.125 Gm.	99
Zinc oxide, 0.750 Gm.	865
Lime water, 2.125 Gm.	60
Zinc oxide, 0.750 Gm; lime water, 2.125 Gm.	349
Olive oil, 2.130 Gm.; lime water, 2.125 Gm.	110
Zinc oxide, 0.750 Gm.; lime water, 2.125 Gm.; olive oil, 2.130 Gm.	185
Zinc oxide, 0.750 Gm.; olive oil, 2.130 Gm.	118
Calamine liniment, N. F. IX	172

Several phenols commonly prescribed in connection with zinc oxide and calamine preparations were incorporated with calamine liniment and irradiated. The phenols used and the percentages are shown in Table II. The samples were irradiated twenty-four hours and seven days after preparation. The results of this study show some interesting facts. Phenol in 2% concentration gave the most peroxide of any phenol used and with all percentages the amount of peroxide gradually increased on aging the liniment for seven days. A 0.1% resorcinol concentration gave more peroxide than did the 1% and 2% quantities. Aging of the resorcinol preparations showed an increase in peroxide but not as much as did the phenol-containing preparations.

TABLE II.—CALAMINE LINIMENTS STUDIED FOR HYDROGEN PEROXIDE FORMATION

Calamine Liniment N. F. IX + 0.1% w/v phenol
Calamine Liniment N. F. IX + 0.1% w/v resorcinol
Calamine Liniment N. F. IX + 0.1% v/v resorcinol monoacetate
Calamine Liniment N. F. IX + 0.1% w/v β-naphthol
Calamine Liniment N. F. IX + 1 % w/v phenol
Calamine Liniment N. F. IX + 1 % w/v resorcinol
Calamine Liniment N. F. IX + 1 % v/v resorcinol monoacetate
Calamine Liniment N. F. IX + 1 % w/v β-naphthol
Calamine Liniment N. F. IX + 2 % w/v phenol
Calamine Liniment N. F. IX + 2 % w/v resorcinol
Calamine Liniment N. F. IX + 2 % v/v resorcinol monoacetate
Calamine Liniment N. F. IX + 2 % w/v β-naphthol

TABLE III.—A SUMMARY OF THE EFFECTS OF CONCENTRATION AND AGE ON THE PEROXIDE FORMATION BY CALAMINE LINIMENT WHEN VARIOUS PHENOLS ARE ADDED TO IT

Phenol Used	Concn., %	Peroxide, μg. 24 hr.	Peroxide, μg. 7 days
Phenol	0.1	181	258
Phenol	1.0	325	430
Phenol	2.0	383	573
Resorcinol	0.1	497	528
Resorcinol	1.0	246	339
Resorcinol	2.0	198	294
Resorcinol monoacetate	0.1	298	424
Resorcinol monoacetate	1.0	206	166
Resorcinol monoacetate	2.0	143	124
β-naphthol	0.1	287	286
β-naphthol	1.0	467	406
β-naphthol	2.0	380	367

Calamine liniment without phenols produced 172 μg. of peroxide.

With resorcinol monoacetate a 0.1% concentration produced the greatest amount of peroxide and this preparation did show an increase on aging. The 1% and 2% concentrations produced less peroxide and these concentrations showed a further decrease on aging. β-Naphthol in 1% concentration gave the highest peroxide values. The aging of all β-naphthol preparations indicated that no significant changes took place.

In order to obtain maximum peroxide values, it appears from the results that when these phenols are used in calamine liniment, phenol should be used in 2% concentration, resorcinol in 0.1%, resorcinol monoacetate in 0.1%, and β-naphthol in 1.0%. These values are different than those found by Mathias and Guth (4), in their studies on calamine lotion. It was shown in their work that a 1% resorcinol produced the greatest amount of peroxide, and that a 1% phenol and a 1% resorcinol monoacetate concentration should be used.

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The Ganglionic Blocking Activity of a Series of 4-Aminoethylpiperidine Derivatives*

By KENNETH I. COLVILLE and ROCCO V. FANELLI

A series of derivatives of 4-aminoethyl piperidine has been tested for ganglionic blocking activity. The most active compound, 1-methyl-4-(pyrrolidinoethyl)-piperidine diethiodide, is approximately equal to hexamethonium in hypotensive and ganglionic blocking potency.

NORTON AND PHILLIPS (1) found 1-methyl-3-(ω-dimethylaminobutyl) piperidine dihydrochloride (BW 51-143) to be equal to hexamethonium in ganglionic blocking potency when given intravenously to laboratory animals and to be better absorbed when administered orally. This high degree of activity in a tertiary amine was somewhat surprising and prompted the study of related compounds. The object of the present experiments was to examine the ganglionic blocking qualities of a series of 4-aminoethylpiperidine derivatives structurally related to BW 51-143.

EXPERIMENTAL

Blood Pressure.—Blood pressure measurements were made in cats of both sexes anesthetized with pentobarbital and chloralose, the cannulated carotid artery being connected to a mercury manometer which recorded directly on a smoked-paper kymograph. Since the extent of the fall in pressure following the administration of hypotensive drugs is dependent to a large degree on the original blood pressure level, the percentage fall rather than the actual drop in mm. Hg was considered to be a more accurate basis for comparison.

Ganglionic Block.—The nictitating membrane of the anesthetized cat was attached to a lever with seven-fold magnification which recorded on a smoked-paper kymograph. Contraction of the nictitating membrane was elicited by an electrical stimulus delivered from a Harvard stimulator to shielded electrodes placed on the preganglionic fibers of the left cervical sympathetic nerve. The stimulus was of 5-volt intensity, 10 cycles per second, and was applied for a duration of six seconds every six minutes. After several control contractions were recorded the compound was administered intravenously two minutes prior to the next stimulation. The smallest of any of the succeeding contractions was used as a basis for calculating the percentage inhibition exerted by the drug.

Acute Toxicity.—Acute toxicity was determined in male albino CF-1 strain mice. Solutions of the drugs were injected into the tail veins at a rate of about 1.0 ml. per minute, the maximum volume injected being 0.35 ± 0.15 ml. The LD_{50} and its standard error were estimated by the method of de Beer (2).

RESULTS AND DISCUSSION

The hypotensive effect of the various compounds after representative dosage is shown in Table I. A possibility always exists that drugs of this type may possess properties other than those causing ganglionic block which will contribute to the depressor response. For example, histamine liberation or central or direct peripheral actions causing vasodilation and/or bradycardia will tend to intensify the blood pressure fall. No evidence for any such extra-ganglionic actions was noted in the present series. The falls in blood pressure elicited by these drugs did not exceed either in extent or duration those caused by hexamethonium at a dosage producing a comparable degree of nictitating membrane block. One member of the series (BW 55-69) was tested on the rat mast cell preparation of Norton (3) and found to liberate histamine to a moderate degree, being in this respect approximately 1 per cent as effective as the highly potent histamine liberator BW 48-80. However, its hypotensive effects in the cat did not appear to be either intensi-

* Received July 3, 1956, from the Wellcome Research Laboratories, Tuckahoe, N. Y.

fied or prolonged and were unaffected by the prior administration of the antihistamine compound chlorcyclizine (Perazil®) at 4 mg./Kg.

Also listed in Table I are the relative ganglionic blocking potencies of the drugs, based on ability to inhibit contraction of the nictitating membrane. All compounds are rated in terms of BW 55-264, the most active of the series, which was arbitrarily assigned a value of 100. BW 55-264 is approximately equal to hexamethonium in ganglionic blocking potency if the two ions are used as a basis for comparison. The diiodide salt used in these studies is, by weight, about one-half as active as hexamethonium chloride.

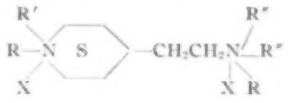
Utilizing the relative potencies as a point of reference it is possible to correlate certain modifications in structure of the quaternary salts with changes in activity. The effect of progressively increasing the size of the quaternizing group R (on both nitrogens) from methyl to ethyl to propyl can be ascertained when the remaining quaternizing groups R′ and R″$_2$N are constant. Similarly, when R and R′ are constant, the effect of enclosing the side chain nitrogen within various heterocyclic groups (R″$_2$N) may be compared.

Two separate pairs of compounds point out the advantages of ethyl over methyl substitution. The diethiodide BW 54-157 is substantially more effective than the corresponding dimethiodide BW 54-141, and the pyrrolidino diethiodide BW 55-264 is also more active than the pyrrolidino dimethiodide BW 54-95. The dipropiodide BW 54-158 is the least potent of all of the quaternary salts and also the most toxic. A comparison of the compounds with heterocyclic substituents in which all remaining quaternizing groups are methyl shows pyrrolidino (BW 54-95) to confer the greatest potency; morpholino (BW 54-95) is least active with piperidino (BW 54-164) intermediate. Pyrrolidino is likewise more effective than alkyl substitution. BW 55-264 was synthesized after preliminary testing had revealed the greater potency coincident with pyrrolidino and ethyl substitution and proved to be the most active of the series.

The tertiary amines as a group display a very low order of activity. It is interesting that the drugs with piperidino and morpholino substituents are more toxic when the nitrogen of the piperidine nucleus is present as a secondary amine (BW 54-143 and 55-69) than when it exists as a tertiary amine (BW 54-202 and 55-100). This increase in toxicity may be due at least in part to histamine release. BW 55-69, as noted above, is moderately active as a histamine liberator.

In the series as a whole a reasonably good correlation appears to exist between toxicity and activity, the most potent drugs in general possessing the lowest LD_{50}. An exception, in addition to the two tertiary amines mentioned above, is the propyl derivative BW 54-158 which is the least effective of the quaternary salts in producing ganglionic blockade and yet is the most toxic. The basis for its lethal action is not known. Mice killed by it die in respiratory failure, yet no neuromuscular block could be demonstrated in the dog sciatic nerve-gastrocnemius muscle preparation at the LD_{50}

TABLE I



BW No.	R″$_2$N	R′	RX	Blood Pressure: Mg./Kg.	Blood Pressure: Fall, %	Blood Pressure: Duration of Fall (min.)	Potency Rating (in Ability to Inhibit Nictitating Membrane Contraction BW 55-264 = 100)	I. V. LD_{50}: Mg./Kg.	I. V. LD_{50}: Limits of Error, %
Quaternary Salts									
55-264	$(CH_2)_4N$	CH_3	C_2H_5I	2	51	60	100	12	90.0–111.2
54-95	$(CH_2)_4N$	CH_3	CH_3I	2	42	40	90	17	88.6–112.8
54-157	$(CH_3)_2N$	CH_3	C_2H_5I	4	48	36	60	19	93.2–107.2
54-164	$(CH_2)_4N$	CH_3	CH_3I	4	33	19	50	18	87.5–114.2
55-95	$(CH_2)_2O(CH_2)_2N$	CH_3	CH_3I	4	24	8	30	26	87.0–115.0
54-141	$(CH_3)_2N$	CH_3	CH_3I	4	24	20	20	40	91.2–109.8
54-158	$(CH_3)_2N$	CH_3	n-C_3H_7I	8	27	17	< 10	8	89.0–112.2
Tertiary Amines									
54-112	$(CH_2)_4N$	CH_3	HCl	8	44	29	30	162	92.3–108.2
54-45	$(CH_2)_4N$	H	HCl	8	47	9	20	185	91.6–109.1
54-143	$(CH_2)_5N$	H	HCl	8	51	7	20	110	90.3–110.7
54-202	$(CH_2)_5N$	CH_3	HCl	8	20	20	10	175	88.7–112.8
55-100	$(CH_2)_2O(CH_2)_2N$	CH_3	HCl	8	10	6	< 10	230	89.0–112.2
54-142	$(CH_3)_2N$	H	HCl	8	23	3	< 10	388	88.0–113.5
54-140	$(CH_3)_2N$	CH_3	HCl	8	24	5	< 10	378	97.0–108.8
55-69	$(CH_2)_2O(CH_2)_2N$	H	HCl	8	21	6	< 10	105	86.5–115.5

dose of 8 mg./Kg. The same dose in anesthetized cats caused no observable effects other than those attributable to a minor degree of ganglionic blockade.

Certain other actions of BW 55-264 have been observed in the anesthetized cat and found to resemble those of known ganglionic blocking compounds. That it also produces parasympathetic block is shown by the fact that 2 mg./Kg. causes a pronounced mydriasis and prevents the blood pressure fall and bradycardia following vagal stimulation. The lack of effect of the same dosage on the depressor action of injected acetylcholine and the similar lack of effect on, or potentiation of, the blood pressure rise following injected epinephrine may be taken as evidence that both its parasympathetic and sympathetic blocking actions are exerted at ganglionic rather than peripheral receptor sites. Like many other quaternaries it is not very well absorbed orally. The ratio of oral to intravenous absorption was obtained by comparing the mouse LD_{50}'s by the two routes of administration. The intravenous/oral ratio for BW 55-264 is 1/31; that for hexamethonium chloride is 1/33.

None of the tertiary amines in the present series approached BW 51-143 in activity. These drugs differ structurally from BW 51-143 in that a two carbon chain is attached at the 4 position of the piperidine nucleus rather than a four carbon chain at position 3; thus five, instead of six, carbon atoms separate the terminal nitrogens. It is possible that this shortening of the carbon chain between the end nitrogens may have contributed to the loss in potency. A similar decrease in ganglionic blocking effectiveness is seen in the polymethylene-bistrimethylammonium series in descending from hexa- to pentamethonium (4). Another possibility is that the point of attachment of the side chain to the piperidine ring may play a decisive role in determining the degree of activity. An examination of other analogs of BW 51-143 should provide at least a partial answer.

SUMMARY

1. The ganglionic blocking potency of a series of 4-aminoethyl piperidine derivatives has been determined.

2. The effect on activity of varying the substituent groups is discussed.

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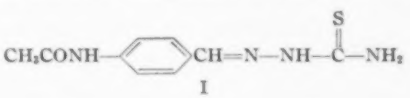
Some Amithiozone (Tibione) Analogs*

By H. CECIL CALDWELL† and W. LEWIS NOBLES‡

The preparation of 12 thiosemicarbazones is reported and the *in vitro* antitubercular activity of many of them, plus others previously reported, is described. The majority of the compounds prepared were monosubstituted derivatives of benzaldehyde 3-thiosemicarbazone, acetophenone 3-thiosemicarbazone or the corresponding α,β-unsaturated derivatives of the above. This latter group comprises vinylogs of the parent compounds. The vinylogs displayed antitubercular activity equal to or greater than that of the parent compounds.

FOR SEVERAL YEARS, the announcement by Domagk and his associates (1) that *p*-acetamidobenzaldehyde thiosemicarbazone, otherwise known as amithiozone or Tibione (I), was



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active against *Mycobacterium tuberculosis* both *in vitro* and *in vivo* has elicited an extensive search for more active and less toxic compounds of this type (2). Results in this area have thus far been rather disappointing, since many compounds, while demonstrating marked activity

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This paper is based on a portion of a thesis submitted by H. Cecil Caldwell in partial fulfillment of the requirements for the Master of Science degree in the Graduate School of the University of Mississippi, June, 1954.
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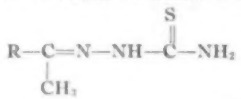
in vitro, either proved to be less active *in vivo* or so toxic that they could not be used clinically.

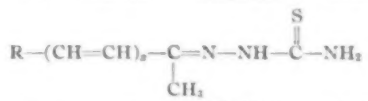
Earlier work (2*e*) had indicated that of the aralkyl ketone thiosemicarbazones studied the thiosemicarbazone of *p*-chloroacetophenone was the most active *in vitro* against the tubercle bacillus. Other workers (2*g*) noted the marked *in vitro* activity of *p*-chlorobenzalacetone thiosemicarbazone. Thus, a vinylog of *p*-chloroacetophenone thiosemicarbazone had demonstrated the same type of physiological activity as had the parent compound. As the similarity in physical properties and chemical reactivity of a compound and its vinylog(s) is well known (3), it would appear that this correlation might be extended to include physiological action in the thiosemicarbazone series. Others (4) have demonstrated this correlation in other areas of medicinal chemistry.

As a consequence we undertook the preparation of a number of thiosemicarbazones in an effort to demonstrate, quantitatively if possible, the relation of the antituberculous activity of a compound of this type and its vinylog.

TABLE I.—KETONES THIOSEMICARBAZONES



No.	R	*x*	Yield, %	M.p.,[a] °C.	Formula	Analyses, %[b] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
1	5-Nitro-2-furyl[c]	...	82	180–182	$C_7H_6N_4O_3S$	36.85	37.14	3.53	3.46

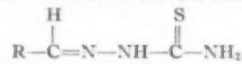


2	2-Thienyl[d]	1	85	125	$C_9H_{11}N_3S_2$	47.97	48.09	4.92	4.97
3	2-Thienyl	2	55	159	$C_{11}H_{13}N_3S_2$	52.56	52.19	5.21	5.39
4	3-Thienyl[d]	1	88	128	$C_9H_{11}N_3S_2$	47.97	48.15	4.92	5.03
5	2-Furyl[c]	1	72	135	$C_9H_{11}N_3OS$	51.67	52.21	5.26	5.16

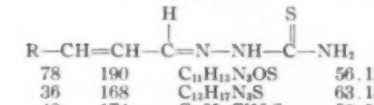
[a] Melting points taken on Fisher-Johns apparatus (uncorrected).
[b] Carbon and hydrogen analyses by Weiler and Strauss, Oxford, England.
[c] Intermediate ketone supplied through the courtesy of Eaton Laboratories.
[d] Intermediate ketones prepared according to the method of H. Keskin, *et al.*, *J. Org. Chem.*, **16**, 199(1951).

The majority of the compounds prepared were monosubstituted derivatives of benzaldehyde 3-thiosemicarbazone, acetophenone 3-thiosemicarbazone or the corresponding α,β-unsaturated derivative of one of the above. Thus, in each case, the vinylene group has been inserted between the aromatic ring and the carbonyl function to produce the vinylog. The nuclear substituents included the CH_3CONH—, $(CH_3)_2N$—, CH_3O—, HO—, CH_3—, *i*-C_3H_7—, and Cl.

TABLE II.—ALDEHYDE THIOSEMICARBAZONES



No.	R	Yield, %	M.p.,[a] °C.	Formula	Analyses, %[b] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
6	2-Chlorophenyl	75	204	$C_8H_8ClN_3S$	44.97	45.34	3.77	3.80



7	4-Methoxyphenyl	78	190	$C_{11}H_{13}N_3OS$	56.12	56.25	5.56	5.69
8	4-Isopropylphenyl	36	168	$C_{13}H_{17}N_3S$	63.13	63.75	6.93	6.61
9	2-Chlorophenyl	42	174	$C_{10}H_{10}ClN_3S$	50.01	50.36	4.21	4.35
10	4-Chlorophenyl	46	178	$C_{10}H_{10}ClN_3S$	50.01	49.78	4.21	4.29
11	4-Methylphenyl	38	158	$C_{11}H_{13}N_3S$	60.24	59.87	5.97	5.91
12	2-Thienyl	90	102	$C_8H_9N_3S_2$	45.48	45.00	4.29	4.47

[a] Melting points taken on Fisher-Johns apparatus (uncorrected).
[b] Carbon and hydrogen analyses by Weiler and Strauss, Oxford, England.

Thiosemicarbazones of a number of heterocyclic aldehydes and ketones together with their vinylogs have also been prepared and are included in this paper.

and stirring was continued for an additional thirty minutes. This mixture was poured on ice and neutralized with sodium hydroxide. The aldehyde was filtered and recrystallized from alcohol twice, using charcoal each time to aid in the decolorization

TABLE III.—THIOSEMICARBAZONES SCREENED IN VITRO AGAINST TUBERCLE BACILLUS H37RV

Parent Compound	Least Amt. of Compound in Mg./100 cc. Which Completely Inhibits Growth in Simple Medium	Single Concn. of 10 mg./100 cc. in Simple Medium Plus 10% Bovine Serum
p-Dimethylaminobenzaldehyde[a]	1.25	No growth
p-Dimethylaminocinnamaldehyde[b]	0.313	No growth
o-Chlorobenzaldehyde[b]	1.25	No growth
o-Chlorocinnamaldehyde[b]	1.25	No growth
p-Chlorobenzaldehyde[a]	1.25	No growth
p-Chlorocinnamaldehyde[b]	1.25	No growth
p-Chloroacetophenone[c]	1.25	No growth
p-Chlorobenzalacetone[d]	1.25	No growth
p-Isopropylbenzaldehyde[a]	0.313	No growth
p-Isopropylcinnamaldehyde[b]	0.313	No growth
p-Tolualdehyde[a]	0.313	No growth
p-Methylcinnamaldehyde[b]	0.313	No growth
2-Thiophenealdehyde[a]	1.00	Not tested
2-Thiopheneacrolein[b]	0.625	No growth
p-(2-Formylvinyl) acetanilide[e]	5.00	No growth
2-Acetylfuran[f]	1.0	Not tested
Furfuralacetone[g]	0.313	No growth
2-Acetylthiophene[a]	4.0	Not tested
4-(3-thienyl)-3-buten-2-one[g]	0.313	No growth
4,4′-Diaminodiphenylsulfone (standard)	2.5	No growth

[a] Prepared according to the method reported by J. Bernstein, *et al.*, *J. Am. Chem. Soc.*, **73,** 906(1951).
[b] See Table II.
[c] Previously reported by W. Lewis Nobles and J. H. Burckhalter, THIS JOURNAL, **42,** 176(1953).
[d] Reported by NG. PH. Boa-Hoi, *et al.*, *J. Org. Chem.*, **18,** 121(1953).
[e] Previously reported by H. Cecil Caldwell and W. Lewis Nobles, *J. Am. Chem. Soc.*, **76,** 1159(1954).
[f] Reported by F. E. Anderson, *et al.*, *J. Am. Chem. Soc.*, **73,** 4967(1951).
[g] See Table I.

EXPERIMENTAL

***p*-Dimethylaminocinnamaldehyde.**—The procedure employed is similar to that of Mohlau and Adam (6), but differs in several important details.

Fourteen and nine-tenths grams (0.1 mole) of *p*-dimethylaminobenzaldehyde was added in small portions, with stirring, to 40 ml. of concentrated sulfuric acid in a 250-ml. flask surrounded by an ice-salt bath, until a clear solution was obtained. Five milliliters of acetaldehyde was added dropwise of the final product. Three and three-tenths grams (20%) of the final product, m. p. 131°, was obtained.

6-(2-Thienyl)-3,5-hexadien-2-one.—Twenty-five grams of β-2-thienylacrolein (0.18 mole), 43 Gm. of acetone (0.33 mole), and 30 ml. of water were treated as described for the preparation of 4-(3-thienyl)-3-buten-2-one (5). Vacuum distillation yielded 16 Gm. (52%) of the desired product, b. p. 130–140° (1–2 mm.).

TABLE IV —THIOSEMICARBAZONES SCREENED IN VITRO AGAINST TUBERCLE BACILLUS H37RV

Parent Compounds	Medium	Days	Mg % Causing Complete Inhibition	Mg % Causing Partial Inhibition	Standard Control (INH), Mg % Complete
p-Tolualdehyde	Serum	12	0.8	0.4	0.0024
	Synthetic	12	1.56	<0.2	...
	Anaerobic	19	1.56	0.4	...
2-Thiophenealdehyde	Serum	12	<0.2	...	0.0024
	Synthetic	12	<0.2	...	...
	Anaerobic	19	25	1.56	...
p-Chlorobenzaldehyde	Serum	12	<0.2	...	0.0024
	Synthetic	12	<0.2	...	...
	Anaerobic	19	<0.2	...	...
p-Dimethylaminobenzaldehyde	Serum	12	0.2	...	0.0024
	Synthetic	12	3.13	<0.2	...
	Anaerobic	19	1.56	<0.2	...

DISCUSSION

All the compounds listed in Table I are ketone thiosemicarbazones. Those in Table II are aldehyde thiosemicarbazones. The various compounds in both tables represent attempts to make available for biological evaluation certain variations of amithiozone (I) and their corresponding vinylogs. A number of the aldehydes and ketones otherwise unavailable were synthesized either by the well-known procedures of the chemical literature or by methods specifically described in the experimental section of this paper.

All the thiosemicarbazones were prepared by the general method previously described (2*e*). The crude product obtained from the reaction of liquid carbonyl compounds was purified by heating with water and filtering while hot, thus removing the excess carbonyl component and any residual thiosemicarbazide. The derivatives of the solid carbonyl compounds were purified by recrystallization from ethanol or dilute ethanol.

Biological Results.—The thiosemicarbazones in Tables I and II, as well as others previously reported, were submitted to Parke, Davis and Company, Detroit, Mich., for *in vitro* screening. The results are indicated in Tables III and IV. Thus, some of the more active compounds, as indicated in Table III, were subjected to intensified *in vitro* screening techniques. These results are indicated in Table IV.

Consequently, in the preparation of these thiosemicarbazones and their subsequent pharmacological evaluation, we have demonstrated that the *in vitro* antitubercular activity of a compound and its vinylog is related. Moreover, it should be emphasized that, in this series when the vinylog is substituted for its parent compound, the activity of the vinylog is equal to, or greater than, that of the parent compound. In no case yet observed has decreased activity been noted in going from the parent compound to its vinylog in this series.

The marked activity of the thiosemicarbazone of 4-(3-thienyl)-3-buten-2-one against the tubercle bacillus and its INH- and Streptomycin-resistant forms has been reported elsewhere (5). In addition, this compound has produced complete inhibition of strains *Streptococcus pyogenes* and *Staphylococcus aureus* at 20γ/ml. and 10γ/ml. concentrations, respectively. This compound also demonstrated amebicidal activity *in vitro* at 1:2,500 dilution.

In addition, certain of these compounds were subjected to other types of biological evaluation. Schistosomiasis studies in mice were conducted with the thiosemicarbazones of *p*-chlorobenzalacetone, 2-furfuralacetone, *p*-dimethylaminobenzaldehyde, and *p*-acetaminocinnamaldehyde. None of these demonstrated marked activity although, in the latter two cases, all the infected animals survived the entire test period.

In vitro amebicidal studies on the thiosemicarbazones of 5-nitro-2-acetylfuran, 4-(3-thienyl)-3-buten-2-one, 3-thenaldehyde, and 5-nitro-2-furfuralacetone indicated that the thiophene derivatives were relatively inactive while the substituted furans demonstrated amebicidal activity at dilutions of 1:1,000 and greater *In vivo* tests were carried out with the latter ones. The results were not promising at levels producing growth suppression.

In addition, the thiosemicarbazones of 5-nitro-2-acetylfuran and 5-nitro-2-thenaldehyde did not inhibit carbonic anhydrase at 1×10^{-5} *M* concentrations.

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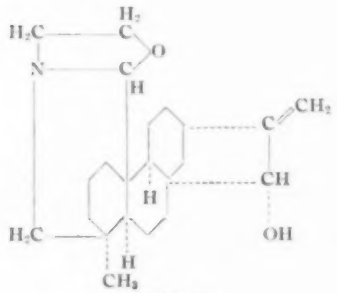
Pharmacological Action of Four *Garrya* Alkaloids*

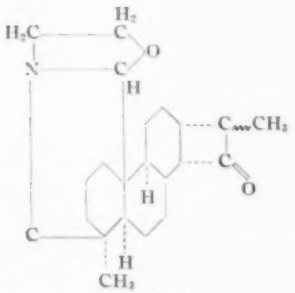
By C. E. POWELL, R. G. HERRMANN, and K. K. CHEN

Four *Garrya* alkaloids, garryine, veatchine, cuauchichicine, and garryfoline, were studied with respect to toxicity and their action on smooth muscle, blood pressure, and respiration. In mice the toxic signs of all four *Garrya* alkaloids were similar. None of the alkaloids had any significantly potent action on smooth muscle organs. Garryfoline seemed to have a weak antihistaminic and anticholinergic action. In the anesthetized cat the *Garrya* alkaloids briefly lowered the blood pressure. Large doses caused respiratory failure. No action was observed on the uterus and intestines *in situ*.

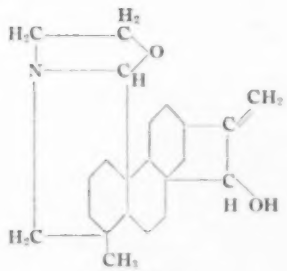
REPORTS in the literature show that extracts of the bark from various *Garrya* species have been used in the treatment of intermittent fever (1) and as a cathartic (2). Two crystalline alkaloids, garryine and veatchine, were isolated by Oneto (1) from *G. veatchii*. Djerassi and his co-workers (3, 4) succeeded in separating two new alkaloids, garryfoline and cuauchichicine, from *G. laurifolia*. The structural relationship of the four *Garrya* alkaloids is as follows:



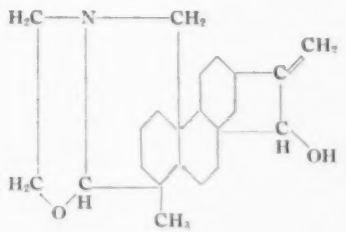
Garryfoline



Cuauchichicine



Veatchine



Garryine

During the war the National Research Council conducted a study on the action of extracts of several *Garrya* species on experimental malaria, and found that garryine was inactive in blood-induced *Lophurae* malaria in ducks (5). It seemed possible, however, that the pure alkaloids might have other important pharmacodynamic activities. Professor Carl Djerassi, Wayne University, Detroit, Mich., kindly supplied us the four alkaloids of *Garrya*, enabling us to make the present investigation.

EXPERIMENTAL

All alkaloids were in the form of their hydrochloride salts except garryfoline, which was an oxalate.

* Received July 16, 1956, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Ind.
The authors wish to thank Dr. Paul N. Harris for the pathological studies.

The acute toxicity was studied in starved albino mice. The compounds were injected via the tail vein, and the animals were observed twenty-four hours for the incidence of death. The concentration of each compound in aqueous solution was adjusted so that the volume injected did not exceed 0.2 ml./Gm. of body weight. The mice receiving garryfoline oxalate were killed and their tissues examined both grossly and microscopically for pathological conditions. The median lethal dose was calculated by the method of Miller and Tainter (6).

The smooth muscle action of these alkaloids was studied on the isolated uterus and ileum of rabbits and guinea pigs. Tissues were suspended in a 10-ml. muscle chamber containing Tyrode's solution maintained at a constant temperature of 37.5°. Responses of the muscle strips were recorded by a gravity writing lever on smoked paper.

The *in vivo* effect of the *Garrya* alkaloids on blood pressure, respiration, uterus, and small intestine was explored in the cat under phenobarbital anesthesia (170 mg./Kg., intraperitoneally). The alkaloids were injected into the exposed femoral vein and responses were recorded in the usual manner.

smooth muscle preparations. With respect to the uteri of both rabbits and guinea pigs, dilutions of 1:10,000 and 1:5,000 of these alkaloids had either no action or only a very weak spastic action. The same concentrations produced either no response or slight relaxation of tone with a minimal decrease in amplitude on small intestine segments of the rabbit and guinea pig. Additional tests with garryfoline oxalate on the ileum of guinea pigs indicated that this alkaloid possessed a weak antihistaminic and anticholinergic action. A dilution of 1:5,000 partially released the spasm induced by 1:10,000,000 dilutions of both histamine and methacholine.

In Vivo Effects.—At a dose of 5 mg./Kg., garryine hydrochloride decreased the blood pressure by 20 mm. Hg for fifteen to thirty minutes and the heart rate by 24 beats per minute, while respiration was slightly increased. Veatchine hydrochloride in doses of 0.5 or 1 mg./Kg. either decreased or increased the blood pressure to a small degree with a slight decrease in heart rate. In one cat a dose of 2 mg./Kg. resulted in death due to respiratory paralysis. Doses of 1 and 5 mg./Kg. of cuauchichicine hydrochloride also lowered the blood pressure for a brief period but increased the heart rate

TABLE I.—ACUTE TOXICITY IN MICE AFTER INTRAVENOUS ADMINISTRATION

Alkaloid	No. Mice Used	LD_{50}, mg./Kg.	Toxic Symptoms
Garryine·HCl	30	12.76 ± 0.45	Increased respiration, clonic convulsions,
Veatchine·HCl	30	12.53 ± 0.75	Increased respiration, clonic convul-ions, gasping.
Cuauchichicine·HCl	9	*	Tremors followed by prostration.
Garryfoline oxalate	42	16.2 + 0.35	Gasping, convulsions, prostration, protrusion and blanching of eyeballs, respiratory failure.

* All animals died on 10 mg./Kg. Because of insufficient material, the LD_{50} was not determined.

RESULTS AND DISCUSSION

Acute Toxicity and Pathology.—The median lethal dose (LD_{50}) of each of the four *Garrya* alkaloids is given in Table I. Their toxic signs were similar. In all cases death appeared to be due to respiratory failure.

Both gross and microscopic examination of the organs from the mice receiving garryfoline oxalate revealed no significant pathologic manifestations. The tissues examined were heart, lung, liver, pancreas, spleen, gastrointestinal tract, kidney, adrenal, and thymus.

Isolated Smooth Muscle Studies.—All of the alkaloids investigated required relatively large doses in order to alter the normal movements of by 20 beats per minute. Following a dose of 10 mg./Kg. the cat died with respiratory failure. No uterine or gut response was observed in the doses given.

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A Comparative *in Vivo* Study of Antacids I*

By ELIAS W. PACKMAN, MORTON E. GOLDBERG, and J. W. E. HARRISSON

This study attempts to draw a comparison between the *in vitro* and *in vivo* antacid properties of dihydroxyaluminum sodium carbonate, aluminum hydroxide, sodium bicarbonate, and calcium carbonate. These agents were administered in various doses to dogs prepared with a simple Pavlov fistula. Thus, the resulting data permit a logical comparison between chemical neutralization and *in vivo* antacid effectiveness.

IN A PREVIOUS REPORT (1) four well-known antacid agents, namely, dihydroxyaluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate were compared by evaluating their ability to reduce the incidence of depot-histamine-induced ulceration in guinea pigs. In the order of decreasing effectiveness the agents were: dihydroxyaluminum sodium carbonate (DASC), sodium bicarbonate, aluminum hydroxide, and calcium carbonate. Histamine-induced ulceration in the guinea pig is due for the most part to the elaboration of a highly acid gastric juice, therefore, the reduction in the incidence of ulceration exhibited by the aforementioned substances is in measure due to their antacid action.

We have therefore, attempted to evaluate these same antacid materials by an *in vitro* method which more closely approximates the *in vitro* tests. The method which we have chosen is one employing the Pavlov simple-gastric-fistula-dog. An earlier report in which DASC was evaluated (2) clearly described the advantages of such a preparation.

Clark and Adams (3) employing Cope pouch dogs, found that sodium bicarbonate and calcium carbonate increased gastric secretion while aluminum hydroxide has no such effect. However, this latter study was mainly interested in the effect on gastric volume, whereas we will be concerned primarily with the control of gastric acidity.

In addition, the authors will attempt to draw a comparison between this latter *in vivo* method and an *in vitro* acid-combining method for antacids described in the U. S. P. XV.

METHODS

Nine mongrel dogs weighing 9 to 12 Kg. were each prepared with a Pavlov simple gastric fistula. A spool-shaped polyethylene cannula of our own design was employed (2).

Food was withheld for eighteen hours prior to each experiment. At this time normal gastric juice secretion was scanty, hence gastric flow was stimulated by the subcutaneous injection of 1.0 mg. histamine diphosphate.

Animals were used but once a week, since it was shown earlier that too frequent administration of histamine causes gastric irritation.

Experiments were scheduled so that each dog received each of the antacids and served as his own control. That is, during the study, the animal would receive histamine followed by one of the four agents or by no antacid at all. This meant that each dog was used 5 times, completing a single dosage regimen of drugs and control.

TABLE I

Antacid	Ml. 0.1 *N* Acid to Neutralize (*p*H 3.8) 1 Gm. of Antacid
DASC	275
Aluminum hydroxide	262
Calcium carbonate	222
Sodium bicarbonate	124

Preparation of Solutions.—Histamine diphosphate was dissolved in distilled water so that the resulting solution contained 1 mg. of material per ml.

Three separate dosages of each of the antacids were compared. Suspensions or solutions of each of the agents containing 0.5, 1.0, and 5.0 grams, respectively, were made in distilled water prior to each day's experiment. The total volume administered was 5 ml.

Sampling of Gastric Secretions.—A gastric juice sample was taken five minutes after the administration of histamine diphosphate. This was considered to be zero time. At this point, the suspended or dissolved antacids were introduced into the stomach via the gastric cannula. Gastric samples were then taken every fifteen minutes for a period covering seventy-five minutes. The volume of juice removed at each sampling was approximately 3 ml. On each sample, free and total titratable acidities were determined by titration with 0.1 *N* NaOH using bromophenol blue and phenol red as indicators, while *p*H measurements were made with a Beckman *p*H meter.

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In Vitro Acid Combining Power.—As directed in the U. S. P. in the monograph for dried aluminum hydroxide gel the following method was employed: 250 mg. of dried antacid was weighed accurately and transferred to a 250-ml. glass-stoppered flask. Exactly 100 ml. of 0.1 *N* hydrochloric acid was added, and the resultant mixture shaken continuously at 37° for one hour. To a 50-ml. aliquot of the solution, bromophenol blue T. S. was added and the excess acid titrated with 0.1 *N* sodium hydroxide to *p*H 3.8.

RESULTS

In Vitro

In vitro acid combining values of the four antacids are presented in Table I.

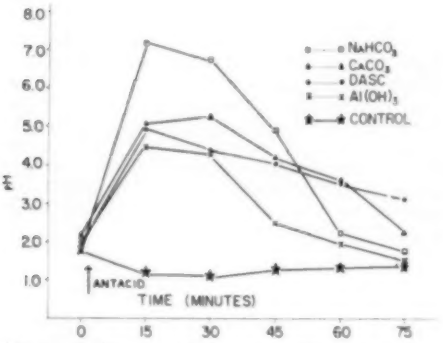

Fig. 1.—Effect of 0.5 Gm. of antacid on *p*H of histamine-induced gastric acid secretion.

In Vivo

DASC in dosages of 0.5, 1.0, and 5.0 grams, respectively, reduced the free acidity of the gastric juice to practically zero within fifteen minutes after administration and the acidity remained at this low level over a sixty-minute period. There was a tendency for the free acidity to increase after sixty minutes, following administration of 0.5 and 1.0 gram of DASC. This increase was approximately twenty per cent of the initial high acid level immediately following histamine injection.

Representative values of *p*H measurements of the gastric samples after DASC administration are presented in Figs. 1, 2, and 3.

Aluminum Hydroxide.—In dosages of 0.5, 1.0, and 5.0 grams, respectively, aluminum hydroxide reduced the free acidity of the gastric juice to practically zero within fifteen minutes after administration and the acidity remained at this very low level over a forty-five-minute period. There was a tendency for the free acidity to increase after forty-five minutes, following administration of 0.5 and 1.0 gram of aluminum hydroxide. This increase approached the initial high acid level at sixty minutes.

Representative values of *p*H measurements of the gastric samples after aluminum hydroxide administration are presented in Figs. 1, 2, and 3.

Calcium Carbonate.—In dosages of 0.5, 1.0, and 5.0 grams, respectively, calcium carbonate reduced the free acidity of the gastric juice to practically zero within fifteen minutes after administration and the acidity remained at this very low level over a forty-five-minute period. There was a tendency for the free acidity to increase after forty-five minutes, following administration of 0.5 and 1.0 gram of calcium carbonate. This increase approached the initial high acid level at sixty minutes.

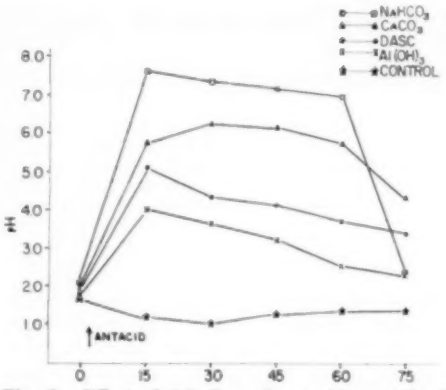

Fig. 2.—Effect of 1.0 Gm. of antacid on *p*H of histamine-induced gastric acid secretion.

Representative values of *p*H measurements of the gastric samples after calcium carbonate administration are presented in Figs. 1, 2, and 3.

Sodium Bicarbonate.—In dosages of 0.5, 1.0, and 5.0 grams, respectively, sodium bicarbonate reduced the free acidity of the gastric juice to practically zero within fifteen minutes after administration. The acidity remained at this low level over a forty-five-minute period after 0.5 gram of sodium bicarbonate was administered, then the free acidity increased to the initial high acid level.

After 1.0 gram of sodium bicarbonate the free acidity remained low over a sixty-minute period; however the seventy-five-minute sample returned to the initial high level of free acid.

Five grams of sodium bicarbonate reduced the

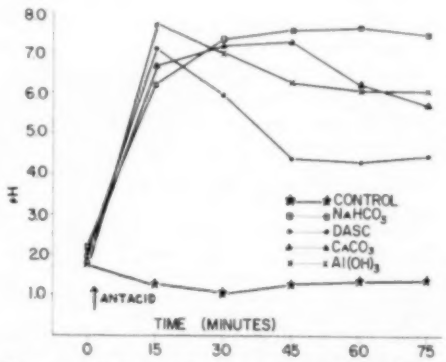

Fig. 3.—Effect of 5.0 Gm. of antacid on *p*H of histamine-induced gastric acid secretion.

free acidity to a level of practically zero over the entire seventy-five-minute period.

Representative values of *p*H measurements of the gastric samples after sodium bicarbonate administration are presented in Figs. 1, 2, and 3.

DISCUSSION

There are two criteria which should be considered when evaluating the efficacy of an antacid. These factors are the neutralization or reduction of the free acid concentration of the gastric juice, and the ability of the antacid to maintain the *p*H of the gastric contents within limits approximating *p*H 3 to 5.5. Further reduction in hydrogen ion concentration would inhibit the physiologic digestive processes of the stomach (4). All the antacids studied reduce the free titratable acidity of the gastric juice to a very low level; however, this alone cannot be used as the criterion of effectiveness since the reduction in free titratable acidity does not always reflect the *p*H.

In comparing these four antacids it is evident that at five-gram doses, each of the agents raised the *p*H of the gastric juice above the desired range. However, only DASC returned the *p*H to the optimum level within the observation period. It may be that the initial rise in *p*H is due to the large dose of material administered, for, actually five grams of an antacid at one administration greatly exceeds dosages employed clinically. The antacid probably exceeds the stimulus during the first few minutes since it has been shown that histamine exerts its maximum effect thirty minutes after injection. Thus, the *p*H values obtained after thirty minutes more clearly demonstrate the value of the antacid at this large dose.

At dosages of 0.5 and 1.0 gram, respectively, only DASC maintained the *p*H of the gastric juice at the optimum level over the entire observation period. Aluminum hydroxide exhibits its effect for forty-five minutes in the optimum range and then the *p*H gradually falls below *p*H 3. Calcium carbonate and sodium bicarbonate at these dosages raise the *p*H above the desired limits initially and either reduce it well below *p*H 3 or maintain it above the optimum level.

Since we must consider both neutralization and *p*H when evaluating an antacid material, it is obvious that the *in vitro* method for determining the acid-combining power of these agents is not indicative of the clinical effectiveness of the antacid. One must also recognize that antacid agents may possess properties in addition to their acid-neutralizing effect, i.e., demulcent action, antipeptic action, or antisecretory action. Demonstration of such properties of antacids has been described by Komarov and Komarov (5), Komarov and Krueger (6), and Schiffrin and Komarov (7). Therefore, it seems essential that antacids be evaluated by *in vivo* techniques and that the *in vitro* tests should be employed only as a rough screening procedure.

On the basis of controlled *p*H and the free titratable acidity achieved with the wide dosage regimen employed, the usefulness of the antacids studied in the order of their decreasing effectiveness is: dihydroxyaluminum sodium carbonate, aluminum hydroxide, calcium carbonate, sodium bicarbonate.

SUMMARY

1. Four well-known antacids have been compared in the gastric fistula dog and by *in vitro* acid-combining power.

2. In a dosage range of 0.5–1.0 gram the antacids in the order of their decreasing effectiveness were: dihydroxyaluminum sodium carbonate, aluminum hydroxide, calcium carbonate, sodium bicarbonate.

3. Over a seventy-five-minute observation period only DASC maintains gastric *p*H in the optimum range of *p*H 3 to *p*H 5.

4. The advantages of this *in vivo* study employing gastric fistula dogs are presented with respect to *in vitro* procedures for evaluating antacids.

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A Comparative *in Vivo* Study of Antacids II*

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The *in vitro* neutralizing power of three commercial antacid tablets was compared with their *in vivo* antacid action as measured by inhibition of experimentally induced gastric ulcers in guinea pigs. The active or principal active ingredients of the tablets were dihydroxyaluminum sodium carbonate, aluminum hydroxide, and calcium carbonate, respectively.

IT HAS BEEN SUGGESTED that an antacid agent loses some of its *in vivo* action when it is compressed into a tablet or upon aging. The purpose of this study is to compare the *in vivo* and *in vitro* antacid effects of three commercially available antacid tablets. By comparing the data of this study with that previously reported (1) upon the active ingredients, or the principal active ingredient of the tablets, we may draw some conclusion as to the effect of tableting on *in vivo* effectiveness. We also desire to stress the importance of a biologic approach in evaluation as compared to the more easily applied, but less important, chemical procedures.

The criterion in this study is the production of gastric ulceration in guinea pigs by the administration of depot histamine (2, 3) and the prevention of this response by the antacid. It is believed that ulcers produced by such a regimen are due for the most part to the elaboration of a copious amount of gastric juice of high acidity. Thus, the protection afforded by these agents may be due in part to their antacid action.

METHODS

English, smooth hair, male, guinea pigs of 250 to 300 Gm. body weight were employed in this study. Gastrointestinal ulceration was produced according to the methods of Hay, *et al.* (2), and the modification of Harrisson, *et al.* (1). The animals received daily an intramuscular injection (into the back musculature) of 50 mg./Kg. histamine diphosphate, while aqueous suspensions of the antacid tablets were administered twice daily via oral intubation. This regimen continued for a ten-day period. All survivors were autopsied on the tenth day and the stomach and duodenum examined for ulceration. Animals which died during the experimental period were autopsied as soon after death as possible. Degree of ulceration (zero to ten) was established according to the evaluation procedure described by Harrisson, *et al.* (1), and the index of ulceration calculated according to the arbitrary standards and formula of Pauls, *et al.* (4).

According to this formula:

$$\text{Index of mucosal damage} = \left(\begin{matrix}\text{Mean degree of}\\ \text{mucosal damage}\end{matrix} \times \begin{matrix}\%\text{ of group with}\\ \text{mucosal damage}\end{matrix}\right) \Big/ 100$$

It is to be noted that the lesser the index of mucosal damage, the more effective the compound.

A second index, which we believe more accurately reflects usefulness, and which takes into consideration the response of the control group has been termed the index of protection. When comparing the index of protection of various antacids, we find that the agent with the highest index is the most effective. This index may be calculated as follows:

$$\text{Index of protection} = \begin{matrix}\text{Index of mucosal damage}\\ \text{(control group)}\end{matrix} - \begin{matrix}\text{Index}\\ \text{of mucosal damage}\\ \text{(experimental group)}\end{matrix}$$

Evaluation of Gastrointestinal Damage.—As mentioned, the mean degree of damage or ulceration was determined by arbitrary standards. Definite values were assigned to the various grades of gastrointestinal damage (Table I); representative degrees of damage are illustrated in Fig. 1 by photographs of guinea-pig stomachs examined during this study.

Preparation of Depot Histamine.—Histamine diphosphate was incorporated in a mineral-oil-beeswax depot as previously described (3). One large batch was prepared since the rate of absorption of depot histamine may vary with each lot. The

TABLE I.—SCORING PROCEDURE

Score	Descriptive Observation*
0	Normal rugal pattern
1	Alteration in normal rugal pattern
2	An occasional or moderate, focal hemorrhagic lesion
4	Severe, focal hemorrhagic lesions (involvement of mucosa, muscularis or serosa)
6	Generalized mucosal hemorrhages
8	Well-defined ulcer
10	Penetrating or perforating ulcer

* All evaluation is based upon macroscopic observation.

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Fig. 1.—Guinea-pig stomachs. *0*, Control Stomach. The rugal pattern is well defined and the gastric mucus is clear and transparent. *2*, Rugal pattern lost in the center of the specimen and is associated with increased vascular congestion of mucosa. Occasional petechial are noted. *4*, Focal mucosal hemorrhages, lesions present surrounded by numerous petechial. Mucosa congestion increased. *6*, Extensive generalized mucosal hemorrhage interposed between flattened, gray, damaged, gastric mucosa, remaining rugal folds accentuated. *8*, Sharply demarcated, well-circumscribed gastric ulcer. Note the almost total absence of rugal and the thin, translucent gastric well. *10*, Oval, circumscribed penetrating gastric ulcer with umbilication and fixation of the serosa. Raised circular ridge around ulcer represents area of fibrosis and healing.

mixture readily melts on a hot plate and can be drawn up into a tuberculin syringe.

An aliquot of previously triturated tablets was used for determination of *in vitro* acid-combining power. The procedure employed was that specified in the monograph for dried aluminum hydroxide gel in the U. S. P. XV (Table II).

TABLE II

Tablet	Av. Wt. of Tablet, Gm.	Acid Consuming Capacity (ml. 0.1 N Acid to Neutralize 1.0 Gm. of Tablet) (pH 3.8)	Acid Consuming Capacity (ml. 0.1 N Acid to Neutralize 1 Tablet) (pH 3.8)
A	1.486	51.0	75.8
B	0.857	101.2	85.0
C	1.271	75.2	95.2

Upon the basis of these results a study was undertaken in which the agents were compared on the basis of their relative acid-combining power. One-quarter of each tablet was suspended in 5 ml. distilled water and the following dosages administered twice daily *per os*, in the morning and evening: tablet A—5 ml., tablet B—4.4 ml., tablet C—4 ml. Control animals received only distilled water at the same time intervals.

A second comparison of the tablets was also made by administering the agents on the basis of equal dosage. One-quarter of each of the tablets was suspended in 5 ml. distilled water and administered twice daily *per os*. Control animals received only distilled water at the same time intervals.

TABLE III.—GASTRIC ULCER; ANTACID STUDY

Depot Histamine 50 mg. i. m. — Antacid Dosage on Basis of Acid Combining Power *per os*, b. i. d.	Tablet A	Tablet B	Tablet C	Control Distilled Water
No. animals with mucosal damage/no. animals in group	8/13	11/13	10/13	13/13
Per cent of animals with mucosal damage	61	85	84	100
Mean degree of mucosal damage	3.3	4.3	4.4	5.7
Index of mucosal damage[a]	2.0	3.7	3.7	5.7
Index of protection[a]	3.7	2.0	2.0	

[a] See text.

RESULTS

Comparison of Three Marketed Antacid Tablets Administered on the Basis of Acid-Combining Power.—The administration of an aqueous suspension of any one of these tablets on the basis of equal neutralization value for ten days partially prevented the mucosal damage produced by depot histamine injection. In the order of *decreasing* effectiveness, the products were: tablet A (active ingredient—dihydroxyaluminum sodium carbonate), tablet B (active ingredient—aluminum hydroxide), tablet C (active ingredient—calcium carbonate-magnesium trisilicate-magnesium carbonate).

The results of this study are summarized in Table III. It may be observed that the *Index of Mucosal Damage* of the control group which received only distilled water was 5.7, while that of the group treated with tablet A was 2.0, after tablet B, 3.7; and after tablet C, 3.7. Comparison of the *Index of Protection* indicates that tablet A (active ingredient—dihydroxyaluminum sodium carbonate) is approximately twice as effective as either of the other two products.

It is interesting to note that only 61 per cent of the animals receiving tablet A exhibited any mucosal damage, while 84 per cent of the animals receiving tablet B or tablet C experienced mucosal damage.

TABLE IV.—GASTRIC ULCER; ANTACID STUDY

Depot Histamine 50 mg. i. m. — Antacids, Equal Doses *per os* b. i. d.	Tablet A	Tablet B	Tablet C	Control Distilled Water
No. animals with mucosal damage/no. animals in group	16/25	21/25	21/25	24/25
Per cent animals with mucosal damage	64	84	84	96
Mean degree of mucosal damage	2.9	4.4	4.4	6.2
Index of mucosal damage[a]	1.9	3.8	3.9	6.0
Index of protection[a]	4.1	2.2	2.1	

[a] See Text.

Comparison of Three Marketed Antacid Tablets Administered on the Basis of Equal Dosage.—The administration of an aqueous suspension containing 1/4 of an antacid tablet twice daily over a ten-day period prevented in a measure the mucosal damage produced by depot histamine injection. In the order of *decreasing* effectiveness, the products were: tablet A (active ingredient—dihydroxyaluminum sodium carbonate), tablet B (active ingredient—aluminum hydroxide), tablet C (active ingredient—calcium carbonate-magnesium trisilicate-magnesium carbonate).

The results of this study are summarized in Table IV. It may be noted that the *Index of Mucosal Damage* of the control group which received no antacid but distilled water was 6.0, while that of the group treated with tablet A was 1.9, after tablet B, 3.8, and after tablet C, 3.9. Employing the *Index of Protection*, tablet A (active ingredient—dihydroxyaluminum sodium carbonate) is approximately twice as effective as either of the other two products.

It is interesting to note that only 64 per cent of the animals receiving tablet A exhibited any mucosal damage, while 84 per cent of the animals

receiving tablet B or tablet C experienced mucosal damage.

DISCUSSION

Production of experimental mucosal damage by the administration of depot histamine is due in a measure to the increased secretion of a highly acid gastric juice. Antacids may work solely by neutralization or they may manifest other properties, i.e., demulcent action, antisecretory action, or antipeptic action.

In attempting to evaluate an antacid, one may be misled by a mere comparison of the numerical values used to designate degree of mucosal damage. For example, if we compare two animals which have been rated as 8 and 4, respectively, at first glance it appears that there is a two to one (2:1) ratio of effect, but this is not necessarily true. A value of 8 represents a well-defined ulcer, while a value of 4 represents only slight mucosal damage and/or hemorrhage. Thus, although there is a 2:1 numerical ratio, the actual "physiologic ratio" may be of the order of ten to one (10:1).

The numerical ratio of tablet A (active ingredient—DASC) to tablet B or C independent of dosages employed in this study is approximately two to one (2:1). However, the index of mucosal damage of the group receiving tablet A indicates that only slight mucosal damage was exhibited by the guinea pigs. In contrast animals in groups receiving either tablet B or C exhibited more extensive mucosal damage. Thus, it appears that tablets of dihydroxyaluminum sodium carbonate are more effective and efficient than tablets of aluminum hydroxide or tablets of calcium carbonate-magnesium trisilicate-magnesium carbonate when compared by this "Depot Histamine-Induced Mucosal Damage in Guinea Pigs."

Comparing this data with that observed in a previous *in vivo* study (1) little or no difference exists between the antacid effects of the tablets and the active ingredient or principal active ingredient which they contain.

We believe it reasonable to conclude that the *in vitro* evaluation of an antacid may only serve as a rough screening procedure.

It has been clearly shown in this study that there is a marked difference in the *in vivo* effects of these agents, while little or no difference is observed when these antacid tablets are compared on the basis of *in vitro* acid-combining power. Thus it would appear that an *in vivo* test for antacid effect more closely approximates the "field conditions" or clinical effectiveness of a compound.

The guinea pig ulceration method may not be the ideal procedure, but whether another animal or method may be superior is a matter of speculation. Of one thing we are certain; that is, the *in vitro* methods for evaluating antacids are not indicative of the clinical value of an antacid agent.

In this study we have seen no evidence that the mechanics of tableting diminish the usefulness of the product. However, we have not investigated any relationship that may exist upon aging, or variations in the rate of tablet disintegration of the different products.

SUMMARY

1. A comparison has been made of an *in vivo* and *in vitro* method of evaluating antacids.

2. Compression of an antacid into a tablet does not appear to diminish its antacid action.

3. On the basis of their decreasing *in vivo* antacid effect, the agents were: tablet A (active ingredient—dihydroxyaluminum sodium carbonate), tablet B (active ingredient—aluminum hydroxide), tablet C (active ingredients—calcium carbonate–magnesium carbonate-magnesium trisilicate).

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Derivatives of alpha-Amino Aldehydes II*

Semicarbazones

By WILLIAM O. FOYE† and WINTHROP E. LANGE‡

Semicarbazone hydrochlorides have been prepared of aldehyde analogs of naturally occurring α-amino acids. These compounds were synthesized by a Rosenmund-type reduction of the α-phthalimido acid chlorides, followed by semicarbazone formation and phthaloyl cleavage using hydrazine. Ten per cent palladium-on-charcoal was found more effective for the reduction than the conventional catalyst. Antiviral testing did not reveal any appreciable activity with these compounds.

SEVERAL ANALOGS of naturally occurring α-amino acids and peptides have been found to exert activity against different types of virus (1). Various aldehyde derivatives such as semicarbazones and thiosemicarbazones have also been shown to possess some antiviral activity (2). The preparation of a series of semicarbazone and thiosemicarbazone derivatives of α-amino aldehydes has accordingly been attempted in order to evaluate their antiviral as well as other biological properties. A series of α-phthalimido aldehyde thiosemicarbazones (3), previously prepared in this laboratory, showed no demonstrable antiviral activity when tested against 100 MLD of encephalitis and influenza viruses in mice; this paper is therefore concerned with the preparation of α-amino aldehyde derivatives containing an unblocked amino function.

Several modifications were employed in the procedure previously reported (3) for α-phthalimido aldehyde derivatives, the most important being the use of thionyl chloride rather than phosphorus pentachloride for the preparation of the α-phthalimido acid chlorides, and the use of 10% palladium-on-charcoal for the reduction of the acid chlorides to aldehydes. With this catalyst and benzene as solvent, a method superior to the conventional Rosenmund reduction of acid chlorides to aldehydes was developed. Yields of pure aldehyde up to 95% were obtained, no catalyst "poison" was required, no evidence of decomposition or reduction to alcohols was noted, a shorter reaction time and lower temperatures were possible, and sulfur-containing compounds (methionine) could be reduced with only partial poisoning of the catalyst. Small amounts (2–3%) of higher melting products which were also obtained were believed to be aldehyde trimers, since they were readily converted to aldehyde derivatives. Further unpublished work by R. Pappo has demonstrated the general utility of this catalyst for reducing acid chlorides to aldehydes.

Three different procedures utilizing palladium-on-charcoal to reduce the acid chlorides were employed, all of which were highly successful. In one, hydrogen was bubbled into a stirred suspension of the catalyst and benzene solution of the acid chloride at reflux temperature. The second procedure was a modification of the first in which the pressure was reduced (the benzene solution refluxed at 38–40°); this shortened the reaction time considerably. The third method consisted of a hydrogenation under pressure, at room temperature, of the acid chloride in ethyl acetate with dimethylaniline as hydrogen chloride acceptor. In the other procedures, the liberated hydrogen chloride was titrated by standard alkali.

The preparation of the semicarbazone derivatives was carried out in accordance with the findings of Conant and Bartlett (4) and of Hammett (5) that this reaction is an acid-catalyzed equilibrium involving free semicarbazide rather than the salt. This necessitated a buffered solution at a *p*H of five; semicarbazide and sodium acetate were accordingly used. When aldehyde trimers were present, refluxing temperatures (in alcohol) were employed for one or two hours before buffering to convert the trimers to monomer. The reaction scheme shown in the previous paper (3) of this series illustrates the formation of the α-phthalimido aldehyde semicarbazones, with the modifications discussed here.

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Abstracted from the dissertation presented to the Graduate School, University of Wisconsin, by W. E. Lange in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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The authors are indebted to Dr. Raphael Pappo for suggesting the catalyst employed in this work.

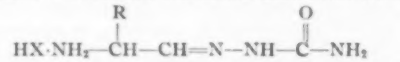
Cleavage of the phthaloyl groups in the α-phthalimido aldehyde derivatives was attempted using hydrazine, and the method of Emerson (6) employing a refluxing aqueous medium was found successful. Using a molar equivalent of hydrazine prevented the formation of an inseparable mixture of hydrazine hydrochloride and amino aldehyde semicarbazone hydrochloride which resulted after acidification when an excess of hydrazine was used. This also permitted isolation of the product by simply evaporating the aqueous solution after removal of phthalhydrazide which precipitates immediately on acidification.

The use of alcohol as solvent for the hydrazinolysis did not result in clear solutions, and made it difficult to determine when the reaction was complete. The slight solubility of phthalhydrazide and the insolubility of the amine salts in alcohol also made it difficult to separate the products. The possibility that the semicarbazone group might be replaced by hydrazone during the reaction is slight. High temperatures and strong alkali are generally required for such a conversion (as in the Wolff-Kishner method of reduction), and a practically quantitative yield of phthalhydrazide was recovered. Elemental analyses of the products also gave final proof of their homogeneity.

indicated the compound to be hydrated, and a Karl Fischer determination verified the presence of two molecules of water of hydration. The aldehyde derivative of methionine was not stable after removal of the phthaloyl group.

The semicarbazone hydrochlorides obtained from valine and phenylalanine were formed with no noticeable differences from the usual reaction, yet analyses of these products gave carbon-hydrogen values that varied considerably from the theoretical. The phthaloyl cleavages were apparently complete, since the calculated amount of phthalhydrazide was isolated. A number of workers (7) have, however, reported difficulty with the elemental analyses of some cyclic cyanogen derivatives, notably the *s*-triazines. These compounds occasionally depolymerize to form cyanogen derivatives with the copper oxide in the carbon-hydrogen combustion train, which are undecomposable at the temperatures customarily employed. Higher temperatures sometimes effect decomposition, however. In view of the propensity of the related amino aldehyde thiosemicarbazones to cyclize to *as*-triazines (see paper III (8) of this series), it is highly possible that the semicarbazones cyclize during combustion to *as*-triazines, thus providing three possible C=N bonds for cyanogen formation. If these groups are eliminated from the

TABLE I.—α-AMINO ALDEHYDE SEMICARBAZONES



R	HX	M. p.[a]	Yield, %[b]	Recrystn. Solvent	Formula	Analyses, %[c] Calcd.	Found
CH_3>$CH-CH_2$ CH_3	HCl	201–202°	62	Abs. ethanol	$C_7H_{17}ON_4Cl$	C: 40.76	40.34
						H: 8.21	8.34
CH_3>CH CH_3	HCl	212–215°	79	Abs. ethanol	$C_6H_{15}ON_4Cl$	C: 37.02	21.31
						H: 7.77	6.97
$C_6H_5-CH_2$	HCl	173–174°	54	Abs. ethanol	$C_{10}H_{15}ON_4Cl$	C: 49.48	28.44
						H: 6.20	6.58
CH_2	$H_2SO_4 \cdot 2H_2O$	208–210°	79	Aq. methanol	$C_4H_{16}O_7N_4S$	C: 18.19	18.00
						H: 6.11	6.60

[a] The melting points were taken on a Fisher-Johns block and are uncorrected.
[b] The yields are based on the quantity of α-phthalimido aldehyde semicarbazone used in the hydrazinolyses.
[c] The analyses were obtained from the Clark Microanalytical Laboratory, Urbana, Ill.

This method of conversion of amino acids to amino aldehyde semicarbazones was successfully carried out with several amino acids, as shown in Table I. The hydrochlorides of the aldehyde semicarbazones derived from glycine and alanine, however, were too hygroscopic for successful isolation and purification. The sulfate of the alanine derivative was found to be fairly stable and purifiable; elemental analysis

theoretical carbon-hydrogen value, a value is obtained more nearly that which was found. Furthermore, one analytical sample of the aldehyde semicarbazone hydrochloride derived from phenylalanine was sent to two different microanalytical laboratories using different combustion methods, and widely varying results were obtained.

Actual proof that the two products in question

have the same structure as those giving correct carbon-hydrogen analyses was obtained through similar ultraviolet absorption spectra, whose characteristics are given in Table II. Further proof was obtained through molecular weight determinations by titration of the hydrochlorides with standard alkali.

TABLE II.—ULTRAVIOLET ABSORPTION SPECTRA OF α-AMINO ALDEHYDE SEMICARBAZONE HYDROCHLORIDES

Amino Aldehyde	λ_{max} (mμ)[a]	ϵ_{max}
2-Amino-4-methylpentanal	231	8,870
2-Amino-3-methylbutanal	233	8,560
2-Amino-3-phenylpropanal	233	8,500
2-Aminopropanal[b]	226	12,300

[a] The spectra were obtained with a Cary Automatic Recording Spectrophotometer using water as solvent.
[b] The sulfate dihydrate was used.

The aldehyde semicarbazone hydrochlorides obtained from leucine and phenylalanine were tested for antiviral activity and found to be ineffective in mice infected with influenza- and encephalitis-type viruses at dosages of 0.5 mg. and 1.0 mg. respectively.[1] The approximate

TABLE III.—INTRAPERITONEAL TOXICITIES OF α-PHTHALIMIDO ALDEHYDE THIOSEMICARBAZONES AND α-AMINO ALDEHYDE SEMICARBAZONE HYDROCHLORIDES IN MICE[a]

Amino Aldehyde Derivative	Toxicity, mg./Kg.
2-Phthalimidoethanal thiosemicarbazone	> 833.0
2-Phthalimidopropanal thiosemicarbazone	>1818.0
3-Phthalimidopropanal thiosemicarbazone	> 41.7
2-Phthalimido-3-phenylpropanal thiosemicarbazone	>1666.0
2-Phthalimido-3-methylbutanal thiosemicarbazone	>1818.0
2-Phthalimido-4-methylpentanal thiosemicarbazone	>1666.0
2-Amino-4-methylpentanal semicarbazone hydrochloride	> 416.5
2-Amino-3-phenylpropanal semicarbazone hydrochloride	> 416.5

[a] Determined at the Lilly Research Laboratories by W. S. Boniece.

intraperitoneal toxicities of these compounds are shown in Table III, where they may be compared with the toxicities of the α-phthalimido aldehyde thiosemicarbazones. It is possible that the phthaloyl group exerts a detoxifying effect; although hydrochloride formation and the resulting increase in solubility may contribute to the greater toxicity of the free amino derivatives. This problem will have to be resolved by further comparisons. It has been observed, however, with another series of compounds (9) that thiosemicarbazones are somewhat, but not appreciably, more toxic than semicarbazones. The much greater toxicity of the β-alanine derivative than of any of the α-amino aldehyde derivatives may be due to a β-type cleavage in the animal body, resulting in reactive, unsaturated compounds. The approximate toxicities reported here should be as meaningful as more precise determinations, since the two classes of compounds would differ in such properties as solubility, rate of solution, and distribution in the animal.

[1] The authors are indebted to Drs. K. K. Chen and W. S. Boniece of the Lilly Research Laboratories for carrying out and reporting the biological determinations.

EXPERIMENTAL

α-Phthalimido Acids.—Phthaloylation of the DL-α-amino acids was carried out according to the general procedures of Ulrich (10) or Billman (11). Yields of 85–95% were obtained.

α-Phthalimido Acid Chlorides.—The acid chlorides were prepared by occasional shaking of a mixture of α-phthalimido acid (0.05 mole) and thionyl chloride (0.1–0.15 mole) on a steam bath for four hours or until solution was complete. The excess thionyl chloride was distilled under reduced pressure. The resulting oils were treated with several 50-ml. portions of benzene, which was removed under reduced pressure, or resulting solids were recrystallized from benzene or Skellysolve C. Acid chlorides which were obtained as solids include those derived from DL-phthaloylphenylalanine, m. p. 130–131°, DL-phthaloylalanine, m. p. 71–71.5°, and phthaloylglycine, m. p. 82–83°. The oil obtained from the reaction with DL-phthaloylmethionine was used immediately after removal of excess thionyl chloride to avoid excessive formation of the cyclic sulfonium salt, which was evidenced by water solubility.

α-Phthalimido Aldehydes.—*Procedure A.*—Dry, thiophene-free benzene (150 ml.), α-phthalimido acid chloride (0.05 mole), and 3 Gm. of 10% Palladium-Darco (American Platinum Works, Newark, N. J.) were placed in a 300-ml. flask equipped with a hydrogen inlet tube and a condenser with an outlet tube submerged in water containing phenolphthalein T. S. The mixture was stirred magnetically and heated by an oil bath at 85° while hydrogen was bubbled in continuously. A rapid evolution of hydrogen chloride took place when the benzene began to reflux. The rate and extent of the reaction were followed by titration of the hydrogen chloride with normal sodium hydroxide solution. After three hours, 90–95% of the theoretical amount of hydrogen chloride had been evolved, and the reaction was stopped. The catalyst was filtered, the benzene was distilled under reduced pressure, and an oil or solid was obtained as in the previous communication (3). Yields of solid product were

93% and 94%, respectively, for the DL-phenylalanine and DL-alanine derivatives.

Procedure B.—This procedure was found particularly suited for the preparation of the phthaloylmethionine aldehyde, which will be used as example. To 150 ml. of dry benzene was added 6.0 Gm. (0.02 mole) of 2-phthalimido-4-methylthiolbutanoyl chloride. The benzene solution was filtered into a 300-ml. flask equipped with a hydrogen inlet tube, thermometer, and condenser with an outlet tube. The outlet tube was inserted in a suction flask also supplied with a 50-ml. buret and containing water and phenolphthalein T. S. The flask was attached to a vacuum pump. Two grams of 10% Palladium-Darco was added to the benzene solution, hydrogen was bubbled through the mixture, and the reaction was stirred magnetically in an oil bath maintained at 60°. Sufficient vacuum was applied to maintain a refluxing temperature of 40°. The rate and extent of the reaction were followed by titration with normal sodium hydroxide solution. After two hours, the reaction was stopped, the catalyst was filtered, and the benzene was distilled under reduced pressure. A yield of 4.8 Gm. of a pale yellow oil was obtained which formed a 2,4-dinitrophenylhydrazone; the oil was used as such for the preparation of the semicarbazone.

Procedure C.—Pure ethyl acetate was prepared by refluxing over calcium hydride for three hours, followed by distillation. The dimethyl aniline was purified by refluxing for three hours over sodium hydride or acetic anhydride. It was then distilled under nitrogen. The 10% Palladium-Darco was dried for two hours at 56° *in vacuo*.

In a 200-ml. pressure bottle were placed 0.01 mole of α-phthalimido acid chloride, 1.0 Gm. of 10% Palladium-Darco, 60 ml. of ethyl acetate, and 2.4 Gm. (0.02 mole) of dimethyl aniline. The mixture was shaken with hydrogen at three atmospheres pressure at room temperature for two hours. At that time, the theoretical amount of hydrogen had been picked up. The catalyst was filtered on Filter-Cel, and the ethyl acetate solution was extracted with several portions of water and one of 5% hydrochloric acid, followed by several portions of 5% potassium carbonate solution and finally water. This removed dimethyl aniline and its hydrochloride as well as unreacted α-phthalimido acid chloride. The ethyl acetate was distilled under reduced pressure, and better than 90% yields of α-phthalimido aldehydes were obtained.

Semicarbazone Formation.—α-Phthalimido aldehyde (0.01–0.05 mole) was dissolved in 50–75 ml. of aqueous ethanol and treated with an equivalent amount of semicarbazide hydrochloride and two equivalents of sodium acetate. The mixture was heated on a steam bath for one hour with occasional swirling, and allowed to cool. The product was filtered and recrystallized from aqueous ethanol. Yields of 85–95% were obtained.

In the reaction with the phthaloylmethionine aldehyde, the mixture was refluxed for two hours before the addition of sodium acetate, and then for an additional hour. After recrystallization from 95% ethanol, a 58% yield of the expected product was obtained which melted at 183–184°.

Anal.—Calcd. for $C_{14}H_{16}O_3N_4S$: N, 17.4. Found: N, 16.8.

Hydrazinolysis.—The procedure of Emerson (6) was modified as follows. Water (100 ml.), α-phthalimido aldehyde semicarbazone (0.02 mole), and either anhydrous hydrazine (0.02 mole) or 85% hydrazine hydrate (0.02 mole) were magnetically stirred in a 250-ml. flask at 100° for three hours. At the end of this time, a pale yellow solution was present. After cooling, the solution was brought to a *p*H of 3 with dilute hydrochloric acid. The precipitate of phthalhydrazide which appeared immediately was filtered, and yields of 80–95% were collected. The aqueous solution was concentrated *in vacuo*, and the residue was dried and recrystallized. Yields of 54–79% were obtained.

In the formation of the alanine derivative (2-amino-propanal semicarbazone sulfate dihydrate), the solution after cooling was acidified with dilute sulfuric acid, filtered, and evaporated to dryness.

Anal.—Calcd. for $C_4H_{12}O_5N_6S{\cdot}2H_2O$: H_2O, 13.64. Found: H_2O, 13.21.

Molecular Weight Determinations.—The following procedure was used to obtain molecular weights of the phenylalanine and valine aldehyde semicarbazone hydrochlorides, which did not give correct carbon-hydrogen analyses. The hydrochloride (0.001 mole) was dissolved in 5 ml. of water followed by the addition of approximately 2 ml. of standardized 0.1 *N* sodium hydroxide solution and a few drops of methyl red T. S. The solution was then titrated with 0.1 *N* sulfuric acid.

Anal.—Calcd. for $C_{10}H_{18}ON_4Cl$: Mol. wt., 242.7. Found: Mol. wt., 240.0.

Anal.—Calcd. for $C_4H_{15}ON_4Cl$: Mol. wt., 194.7. Found: Mol. wt., 193.1.

Determination of Antiviral Activities.—The compounds tested were injected intraperitoneally into mice which were then exposed to 50–75 MLD of virus. Five mice were employed with each compound. Survival of the mice was the criterion for positive activity.

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Esters of Bicyclic Aminoalcohols as Potential Antispasmodics I*

The Synthesis of 2-Hydroxyoctahydroquinolizine and Some of its Esters

By HAROLD J. RHODES† and TAITO O. SOINE

A convenient synthesis of 2-hydroxyoctahydroquinolizine has been described. A number of improvements and modifications of Clemo's original synthesis of 2-keto-octahydroquinolizine are described and have materially contributed to a much higher yield. The reduction of the amino ketone to the previously unknown amino alcohol is described in detail together with the properties of the latter. 2-Chloro-octahydroquinolizine was prepared and used in two unsuccessful attempts to esterify xanthene-9-carboxylic acid, employing the free acid and the silver salt of the acid, respectively. Twelve esters, each in the form of the hydrochloride and methobromide salts, were prepared from 2-hydroxyoctahydroquinolizine and the following substituted acetic acids: (*a*) diethylacetic, (*b*) phenylethylacetic, (*c*) phenylacetic, (*d*) cyclohexylacetic, (*e*) benzylacetic, (*f*) hexahydrobenzylacetic, (*g*) diphenylacetic, (*h*) dicyclohexylacetic, (*i*) dibenzylacetic, (*j*) dihexahydrobenzylacetic, (*k*) fluorene-9-carboxylic, and (*l*) xanthene-9-carboxylic acids. Pharmacological testing indicated that the methobromide salts of esters obtained from phenylethylacetic, benzylacetic, and diphenylacetic acids had high ganglionic blocking activity. Diethylacetic, phenylacetic, and xanthene-9-carboxylic acids had a moderate ganglionic blocking action. The only compound with significant atropine-like activity in the series was the methobromide of the ester derived from xanthene-9-carboxylic acid. This compound appears to combine both types of activity in significant amounts.

ATROPINE has long been known as an antispasmodic of considerable merit but it has also been recognized as having drawbacks because of the multiplicity of its actions. For this reason, much research has been directed toward modification of its structure in an effort to emphasize the desirable qualities and to minimize the undesirable actions. That this research has succeeded in some measure is attested to by the large group of synthetic antispasmodics on the market today. Nevertheless, an ever increasing number of publications and patents describe additional syntheses of active compounds.

The trend in the synthesis of antispasmodic esters usually has been toward the use of disubstituted acetic acids combined with acyclic dialkylaminoalkanols. It is an established fact that a simple alcohol such as 2-diethylaminoethanol does not abolish antispasmodic activity but it is also recognized that a more complex alcohol such as tropine may confer a quantitatively greater activity. Some esters of monocyclic amino alcohols have appeared on the market (e. g., Dactil®, Piptal®, eucatropine) but, by far, the majority of useful compounds are derived from acyclic amino alcohols. Generally speaking, with the exception of products derived from naturally-occurring compounds (e. g., atropine, scopolamine), the examination of esters of synthetic bicyclic amino alcohols has been only sporadic. Sternbach and Kaiser (1) found highly active antispasmodics among the esters of 3-quinuclidinol and 1-azabicyclo[3,2,1]-6-octanol and suggested the further advantage that esters of this type would be more stable to hydrolysis than primary alcohol esters. The object of the present work has been to prepare and examine some of the esters obtainable from the bicyclic amino alcohol, 2-hydroxyoctahydroquinolizine (1-azabicyclo[4,4,0]-3-decanol), to determine whether they possessed significant antispasmodic activity.

A survey of the literature indicated that 2-hydroxyoctahydroquinolizine had not been synthesized.[1] The synthesis of 2-keto-octahydroquinolizine (1-azabicyclo[4,4,0]-3-decanone), however, had been previously reported by Clemo and co-workers (2) but a repetition of their work led to the conclusion that the reported yields were exceedingly difficult to attain. The following synthetic sequence was found to be not only productive of higher yields but decidedly more convenient as well.

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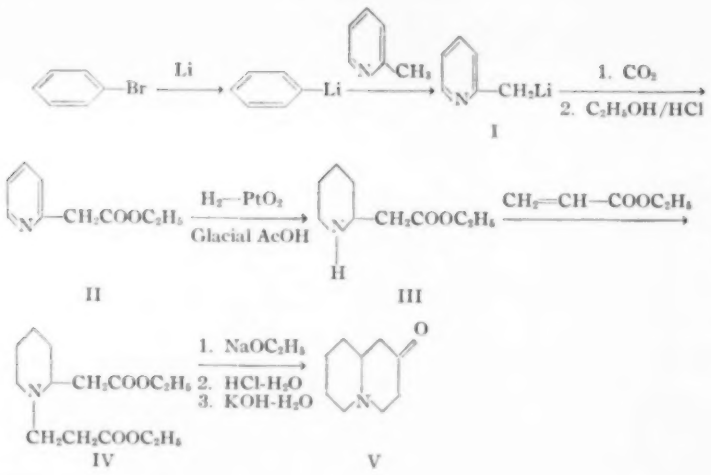
[1] Subsequent to completion of this investigation, V. Boekelheide and W. G. Gall (*J. Am. Chem. Soc.*, **76**, 1832 (1954)) reported another synthesis for this compound. Their reported melting point agreed with ours.



Hydrogenation of 2-keto-octahydroquinolizine, either in the form of the unstable base in glacial acetic acid or as the stable hydrochloride (3) in water, in the presence of platinum oxide and ferrous sulfate yielded the desired amino alcohol in excellent yields. The latter method was preferred because it was more convenient to isolate the amino alcohol and also because the amino ketone hydrochloride was much more stable under storage than the free base.

The second phase of this investigation entailed the preparation of a series of esters of 2-hydroxy-octahydroquinolizine. The acids employed are listed in Table I. The cycloaliphatic acids were prepared by hydrogenation of the corresponding aromatic acids in the presence of platinum oxide catalyst in glacial acetic acid. It was found that the purity of the aromatic acids was of vital importance because hydrogenation could not be effected otherwise. Of the three esterification methods investigated, the acid-chloride-alcohol method was chosen in preference to either the Horenstein-Pählicke or silver salt methods because of convenience in procedure and superior yields. 2-Chloro-octahydroquinolizine, prepared in the usual way, was employed in the investigation of the Horenstein-Pählicke and silver salt methods.

With the acid chloride-alcohol method, variation of the relative quantities of the reactants and reflux periods in anhydrous benzene indicated that if the equivalent quantity of the amino alcohol used was twice that of the acid chloride, the yield of amino ester exceeded 70% and the optimum reflux period was decreased from fifteen to twenty hours to five to eight hours. Sterically-hindered acid chlorides required longer reflux periods than did the relatively unhindered ones. The yields of amino ester were found to be calculable on the basis of the total weight of dried nitrogen-containing residue isolated according to the procedure described in the experimental portion of this paper. Chromatographic separation of the nitrogen-containing residue verified the validity of this method, inasmuch as the quantities of amino ester and unreacted amino alcohol obtained agreed with the calculated ones. Isolation of the esters by means of fractional vacuum distillation, fractional extraction, or fractional crystallization resulted in much lower yields than those obtained by chromatographic means.

The hydrochloride and methobromide salts of each ester were prepared by the usual methods and provided crystalline products with the exception of octahydroquinolizin-2-yl fluorene-9-carboxylate hydrochloride which was an oil.

The hydrochloride and/or methobromide salts of the amino esters were screened for ganglionic blocking activity and anticholinergic, neuroeffector blocking activity.[2] The results are shown in Table II. Relative activity values are also included for Banthine® and Probanthine®, the methobromide salts of the xanthene-9-carboxylates of 2-diethylaminoethanol and 2-diisopropylaminoethanol, respectively.

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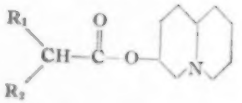
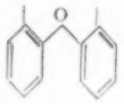
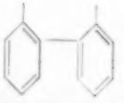
[2] The authors are indebted to Dr. Raymond M. Dodson of G. D. Searle and Co., Chicago, Ill., for the pharmacological data reported in Table II.

TABLE—I



R_1	R_2	Amine Ester[a] Yield, %	Formula Empirical	Melting Point, °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Chloride Calcd.	Chloride Found
C_2H_5—	C_2H_5—	83.5	$C_{15}H_{27}NO_2 \cdot HCl$[a]	159 –160	62.6	62.9	9.7	9.4	4.8	4.7	...	...
			$C_{15}H_{27}NO_2 \cdot CH_3Br$[b]	251.5–252.5	55.2	55.0	8.7	8.8	3.9	4.0	...	...
C_6H_5—	C_2H_5—	83.1 (92.5)	$C_{19}H_{27}NO_2 \cdot HCl$[c]	173 –174	67.5	66.6	8.4	8.5	4.1	4.0	...	...
			$C_{19}H_{27}NO_2 \cdot CH_3Br$[c]	222 –223	60.6	60.6	7.6	7.7	3.5	3.4	...	...
C_6H_5—	H—	66.2	$C_{17}H_{23}NO_2 \cdot HCl$[a]	166 –167	65.9	65.3	7.8	7.9	4.5	4.3	11.4	11.4
		(92.1)	$C_{17}H_{23}NO_2 \cdot CH_3Br$[b]	203 –204	58.7	58.1	7.1	7.2	...	...	...	...
C_6H_{11}—	H—	81.2	$C_{17}H_{29}NO_2 \cdot HCl$[d]	154 –155	64.6	64.3	9.6	9.6	...	...	...	...
			$C_{17}H_{29}NO_2 \cdot CH_3Br$[b]	244 –245 (dec.)	57.7	57.2	8.6	8.3	3.8	4.0	...	...
$C_6H_5CH_2$—	H—	67.3 (94.5)	$C_{18}H_{25}NO_2 \cdot HCl$[a]	153 –154	66.7	66.7	8.1	8.2	4.3	4.2	11.0	11.0
$C_6H_{11}CH_2$—	H—	84.7	$C_{18}H_{25}NO_2 \cdot CH_3Br$[b]	199 –200	59.7	58.6	7.4	7.2	3.7	3.4	...	...
			$C_{18}H_{31}NO_2 \cdot HCl$[d]	182.5–183.5	65.5	65.7	9.7	9.4	4.2	4.2	10.7	10.8
C_6H_5—	C_6H_5—	63.5	$C_{18}H_{31}NO_2 \cdot CH_3Br$[e]	205 –206	58.7	58.2	8.8	9.1	3.6	3.6	...	...
			$C_{23}H_{27}NO_2 \cdot HCl$[a]	210 –211	71.6	71.8	7.3	7.5	3.6	3.6	...	...
			$C_{23}H_{27}NO_2 \cdot CH_3Br$[b]	239.5–240.5 (dec.)	64.9	64.9	6.8	6.8	...	...	...	...
C_6H_{11}—	C_6H_{11}—	62.3	$C_{23}H_{39}NO_2 \cdot HCl$[a]	190 –200	69.4	69.7	10.1	10.3	3.5	3.3	...	...
			$C_{23}H_{39}NO_2 \cdot CH_3Br$[b]	253 –254 (dec.)	63.1	62.6	9.3	9.4	3.1	3.0	...	...
$C_6H_5CH_2$—	$C_6H_5CH_2$—	71.0	$C_{25}H_{31}NO_2 \cdot HCl$[a]	159.5–160.5	72.5	72.6	7.8	7.9	...	...	...	...
$C_6H_{11}CH_2$—	$C_6H_{11}CH_2$—	52.3	$C_{25}H_{31}NO_2 \cdot CH_3Br$[b]	214.5–215.5	66.1	65.9	7.3	7.2	...	...	...	...
			$C_{25}H_{43}NO_2 \cdot HCl$[a]	231.5–232.5	70.5	70.6	10.4	10.4	...	...	...	...
			$C_{25}H_{43}NO_2 \cdot CH_3Br$[b]	227 –228	64.4	63.7	9.6	9.8	2.8	2.5	...	...
		71.7	$C_{23}H_{25}NO_3 \cdot HCl$[a]	258 –259	69.1	68.7	6.6	6.6	...	...	...	...
			$C_{23}H_{25}NO_3 \cdot CH_3Br$[f]	252 –253	62.9	62.9	6.2	6.1	...	...	...	...
		79.4	$C_{23}H_{25}NO_2 \cdot CH_3Br$[c]	216 –217 (dec.)	65.2	65.8	6.4	6.2	3.1	3.0	...	...
			$C_{23}H_{25}NO_2 \cdot HCl$	Oil	Could not be analyzed.							

Solvents used for recrystallization: [a] Acetone-petroleum ether. [b] Isopropyl alcohol-ether. [c] Methyl isobutyl ketone-absolute ethanol. [d] Methyl isobutyl ketone-petroleum ether. [e] Methyl isobutyl ketone. [f] Isopropyl alcohol-ethanol-ether.

[g] The figure in brackets represents the calculated yield (see text) and the other is the isolated yield.

TABLE II.—PHARMACOLOGICAL DATA

Acids Esterified with 2-Hydroxyoctahydroquinolizine	Salt	Activity	
		Ganglion Blocking[a]	Anticholinergic, Neuroeffector Blocking[b]
Diethylacetic	HCl	Approx. 0.1	<0.005 (inactive)
	CH_3Br	1.56 ± 0.12	<0.005 (inactive)
Phenylethylacetic	HCl	.....	<0.005 (inactive)
	CH_3Br	4.44 ± 0.31	<0.005 (inactive)
Phenylacetic	HCl	<0.1	<0.005 (inactive)
	CH_3Br	1.33 ± 0.11	<0.005 (inactive)
Cyclohexylacetic	HCl	<0.4	<0.005 (inactive)
	CH_3Br	.....	<0.005 (inactive)
Benzylacetic	HCl	<0.2	<0.005 (inactive)
	CH_3Br	2.31 ± 0.2	<0.005 (inactive)
Hexahydrobenzylacetic	HCl	.....	<0.005 (inactive)
	CH_3Br	.....	<0.005 (inactive)
Diphenylacetic	HCl	<0.1	<0.005 (inactive)
	CH_3Br	4.32 ± 0.37	<0.005 (inactive)
Dicyclohexylacetic	HCl	.....	<0.004 (inactive)
	CH_3Br	<0.1	0.02 ± 0.003
Dibenzylacetic	HCl	<0.1	<0.004 (inactive)
	CH_3Br	0.49 ± 0.1	<0.005 (inactive)
Dihexahydrobenzylacetic	HCl	<0.1	<0.005 (inactive)
	CH_3Br	<0.1	<0.006 (inactive)
Xanthene-9-carboxylic	HCl	<0.1	0.02 ± 0.004
	CH_3Br	1.40 ± 0.24	0.19 ± 0.05
Fluorene-9-carboxylic	HCl	....	.....
	CH_3Br	<0.1	<0.005 (inactive)
Banthine	...	1.0	1.0
Probanthine	...	2 to 5	1.5

[a] These are rated against a value of 1 for tetraethylammonium bromide.
[b] These are rated against a value of 1 for atropine sulfate.

EXPERIMENTAL[3]

Ethyl Pyridyl-2-acetate (II).—Modification of the method of Woodward and Kornfeld (4) resulted in a more convenient procedure as well as an increase in yield of product. The modifications were: (*a*) the use of an anhydrous nitrogen atmosphere while cutting and introducing lithium into anhydrous ether, (*b*) the transfer of α-picolyllithium to a dropping funnel, previously flushed with anhydrous nitrogen and under reduced pressure, while maintaining a continuous nitrogen atmosphere in the flask from which the transfer was being effected. This prevented crusting due to exposure of α-picolyllithium to atmospheric oxygen and carbon dioxide and the subsequent addition step was not hampered by clogging of the dropping funnel, (*c*) the slow, dropwise addition of α-picolyllithium in anhydrous ether, while under a continuous nitrogen atmosphere, to a vigorously stirred slurry of pulverized "dry ice" in anhydrous ether (5) (maintaining an excess of carbon dioxide at the site of reaction minimized the formation of the undesired side-products, α,α′-dipicolylketone and α,α′,α″-tripicolylcarbinol), and (*d*) the use of Drierite® during the esterification of lithium α-picolinate with absolute ethanol and anhydrous hydrogen chloride.

From 30.0 Gm. (4.33 atomic equivalents) of lithium, 210 cc. (314 Gm., 2.00 moles) of bromobenzene, and 209 cc. (197 Gm., 2.12 moles) of α-picoline, there was obtained 65.4 Gm. (50.8% of theory, based on bromobenzene) of ethyl pyridyl-2-acetate distilling at 128–130°/18 mm.

The picrate of ethyl pyridyl-2-acetate was prepared by adding a slightly less than equivalent amount of ethereal picric acid to an ether solution of the amine. The yellow precipitate was then washed several times with anhydrous ether and recrystallized twice from 95% ethanol. The long, yellow needles melted at 138–139° (4).

Anal.[4]—Calcd. for $C_{15}H_{14}N_4O_9$: C, 45.7; H, 3.6. Found: C, 45.7; H, 3.9.

Ethyl Piperidyl-2-acetate (III).—Modification of the method of Clemo (6) increased the yield of ethyl piperidyl-2-acetate from 72% to virtually a quantitative yield.

Platinum oxide (0.6 Gm.) was added to a solution of ethyl pyridyl-2-acetate (90.2 Gm.) in glacial acetic acid (120 cc.) and the mixture was then subjected to hydrogenation in a Parr low pressure hydrogenator (initial hydrogen pressure, 59 p. s. i.). After three hours, an additional 0.4 Gm. of platinum oxide was added and the hydrogen pressure increased from 27 to 50 p. s. i. The pressure was increased twice more before hydrogen absorption ceased. Approximately 1.64 moles of hydrogen were absorbed over a period of eighteen hours. The catalyst was filtered off and, after removal of the glacial acetic acid by distillation under reduced pressure, the remaining syrupy residue was basified with aqueous potassium hydroxide (10% w/v) and solid potassium carbonate. The basic residue was then extracted with ether and chloroform until the extracts gave a negative test with Valser's reagent (mercuric iodide T.S.). After drying the combined ether and chloroform extracts over anhydrous sodium sulfate and Drierite, the solvents were distilled off and the remaining residue was fractionated

[3] All melting points are uncorrected.

[4] All analyses were performed by the Microanalytical Laboratories of the Department of Chemistry, University of Minnesota, or the Clark Microanalytical Laboratory, Urbana, Ill.

under reduced pressure. A viscous, colorless liquid, which solidified on cooling, was obtained in a yield of 91.8 Gm. (97.8% of theory) at 104°/13.6 mm. and 106°/14.5 mm. Addition of ethereal picric acid solution to an ether solution of the base yielded yellow needles which melted at 126–127° after two recrystallizations from 95% ethanol. The product obtained by Clemo, *et al.*, distilled at 105°/14 mm. and yielded a picrate, m. p. 125°.

Ethyl Piperidyl-2-acetate-1-β-propionate (IV).—The method employed by Clemo, *et al.* (7), to synthesize this compound in 63% yield entailed heating a mixture of ethyl piperidyl-2-acetate, ethyl β-chloropropionate, fused sodium acetate and barium carbonate. The reaction of ethyl β-chloropropionate with ethyl piperidyl-2-acetate presumably involves a dehydrohalogenation of the former to ethyl acrylate, which then undergoes a Michael-type condensation with the latter. Employing ethyl acrylate instead of β-chloropropionate, according to a similar procedure by Mozingo and McCracken (8), not only increased the yield but also provided a more convenient method of synthesis.

Ethyl piperidyl-2-acetate (50.0 Gm., 0.29 mole) was dissolved in absolute ethanol (40 cc.) and added slowly with stirring to a solution of ethyl acrylate (31.5 Gm., 0.315 mole) in absolute ethanol (50 cc.). The reaction mixture was allowed to stand for a period of not less than eight days, correspondingly lower yields being obtained with shorter standing periods. After removal of the solvent by distillation, the orange-colored liquid residue was fractionated under reduced pressure. A forerun, consisting of ethyl piperidyl-2-acetate, distilled at 106–7°/15 mm. A second fraction, consisting of ethyl piperidyl-2-acetate-1-β-propionate (63.0 Gm., 79.8% of theory), distilled over at 161°/6.5 mm., 164°/9.2 mm. and 174°/16 mm. Clemo, *et al.*, reported a boiling point range of 165–169°/11 mm. for ethyl piperidyl-2-acetate-1-β-propionate. No solid derivatives of this diester are described in the literature. All attempts to prepare the hydrochloride, picrate, styphnate, picrolonate, or chloroplatinate derivative yielded syrupy products which resisted crystallization. The diester was then redistilled and submitted for analysis.

Anal.—Calcd. for $C_{14}H_{25}NO_4$: C, 61.9; H, 9.3; N, 5.2. Found: C, 60.9; H, 9.3; N, 5.3.

2-Keto-octahydroquinolizine (V).—Clemo, *et al.* (2), prepared this compound in 36.4% yield by cyclization of methyl piperidyl-2-acetate-1-β-propionate with powdered potassium and subsequent decarboxylation with hydrochloric acid. The authors obtained the same yield of the amino ketone by employing the corresponding diethyl diester. However, when cyclization was effected by means of sodium ethoxide under a nitrogen atmosphere (9), the yield of amino ketone was nearly doubled.

Dry nitrogen was bubbled through xylene (80 cc.) contained in a 0.5 liter, 3-necked, round bottom flask, equipped with a mechanical stirrer, dropping funnel, reflux condenser fitted with a calcium chloride drying tube and a distilling side-arm. Sodium (2.6 Gm., 0.113 gram-atom) was added to the xylene and pulverized by stirring vigorously at a temperature above its melting point. With continued stirring the reaction mixture was allowed to cool to 70°, and then absolute ethanol (6 Gm., 0.13 mole) was added slowly (0.5 hr.). After addition was completed, the temperature was increased to 130° and maintained for one hour. A solution of ethyl piperidyl-2-acetate-1-β-propionate (30.0 Gm., 0.111 mole) in xylene (120 cc.) was then added dropwise at a rate sufficient to maintain a continuous distillation of the freshly formed ethanol (1.5 hr.). Heating while stirring was continued for an additional 0.5 hr. Passage of dry nitrogen was discontinued after the reaction mixture had been allowed to cool to room temperature. The continuously-stirred reaction mixture was then chilled in an ice-salt bath and acidified slowly with cold concentrated hydrochloric acid (23.0 cc.). Stirring was continued for 0.5 hr., after which the xylene and aqueous phases were separated. The xylene phase was extracted with concentrated hydrochloric acid (two 25-cc. portions); the quantities and concentration of hydrochloric acid employed were calculated to yield a solution of 2-keto-3-carbethoxyoctahydroquinolizine hydrochloride in 20% (w/w) hydrochloric acid. The combined acid extracts were then extracted with ether (two 30-cc. portions) to remove traces of dissolved xylene. After removal of the dissolved ether by heating on a steam bath, the acid extract was refluxed until a drop of the solution failed to give a violet color when added to a 1% solution of ferric chloride (2 hr.). The aqueous solution was evaporated to dryness under reduced pressure, and the remaining brown, crystalline residue was basified with a cold aqueous solution of potassium hydroxide (50% w/v). The basic solution was immediately extracted with ether (five 25-cc. portions) and chloroform (two 50-cc. portions). The extracts were washed with a saturated solution of sodium chloride to remove traces of potassium hydroxide and then combined and dried over anhydrous sodium sulfate and Drierite. After removal of the solvents by distillation, 2-keto-octahydroquinolizine was obtained as a colorless liquid distilling at 115°/16.5 mm. (11.5 Gm., 67.8% of theory). The product turned brown in color on standing a few hours. The picrate salt was prepared in the usual manner and was crystallized from 95% ethanol (needles) or acetone (prisms) with either form melting at 209–210°. Clemo and co-workers described the product as a colorless liquid, b. p. 70–72°/1 mm., which changed to a light brown color on standing and formed a picrate, m. p. 211°. After three recrystallizations from 95% ethanol, a sample of the amino ketone picrate was dried under reduced pressure in an Abderhalden drying pistol at 140° for eight hours over phosphorus pentoxide and then submitted for analysis.

Anal.—Calcd. for $C_{15}H_{18}N_4O_8$: C, 47.1; H, 4.7. Found: C, 47.4; H, 4.8.

2-Hydroxyoctahydroquinolizine.—*Procedure A.*—2-Keto-octahydroquinolizine hydrochloride (27.0 Gm., 0.142 mole), prepared by treatment of an ethereal solution of freshly distilled 2-keto-octahydroquinolizine with ethereal hydrogen chloride, was dissolved in water (200 cc.) and reduced in a Parr low pressure hydrogenator at room temperature in the presence of platinum oxide (0.4 Gm.) and exsiccated ferrous sulfate (0.1 Gm.) at an initial pressure of 60 p. s. i. After the calculated amount of hydrogen had been absorbed (six hours), the catalysts were filtered off and the water was re-

moved by distillation under reduced pressure. The remaining white crystalline residue was basified with an ice-cold aqueous solution of potassium hydroxide (50% w/v). Potassium chloride was then added in excess to the basic solution in order to "salt out" the water-soluble amino alcohol. After the basic solution had been extracted with ether (six 25-cc. portions), the extracts were combined and dried over anhydrous sodium sulfate and Drierite. The ether was distilled off from the filtered solution and the remaining light amber-colored residue was then fractionated under reduced pressure. A colorless, viscous liquid (20.5 Gm., 90% of theory) distilled over at 132–133°/15 mm. and solidified to a white wax-like solid on cooling to room temperature. Crystallization of the solid from petroleum ether (Skellysolve B) yielded either colorless rods (slow cooling) or white needles in tufts (rapid cooling), m. p. 91–92°. The amino alcohol was recrystallized twice more from petroleum ether (Skellysolve B), dried over phosphorus pentoxide at room temperature under reduced pressure for eight hours, and submitted for analysis. The melting point remained unchanged.

Anal.—Calcd. for $C_9H_{17}NO$: C, 69.6; H, 11.1. Found: C, 69.4; H, 11.3.

Procedure B.—Freshly distilled 2-keto-octahydroquinolizine (25.6 Gm., 0.167 mole) was dissolved in glacial acetic acid (160 cc.) and reduced in a Parr low pressure hydrogenator at room temperature in the presence of platinum oxide (1.0 Gm.) and exsiccated ferrous sulfate (0.1 Gm.) at an initial pressure of 59 p. s. i. After the calculated amount of hydrogen had been absorbed (3.5 hr.), the hydrogenation mixture was filtered. Addition of ethereal hydrogen chloride to the filtrate yielded a white precipitate which was filtered off, washed with anhydrous ether and dried (31.5 Gm.). The precipitate was then dissolved in water (30 cc.) and basified with a cold aqueous solution of potassium hydroxide (50% w/v). From this point on Procedure B was identical with Procedure A. The melting point of this product was not depressed by admixture with the product from Procedure A so they were assumed to be identical.

2 - Chloro - octahydroquinolizine.—Anhydrous hydrogen chloride was bubbled into a 250-cc. flask containing a solution of 2-hydroxyoctahydroquinolizine (5 Gm.) in chloroform (30 cc.) until precipitation ceased. A dropping funnel containing thionyl chloride (25 Gm.) and a reflux condenser equipped with a calcium chloride drying tube were attached to the flask and the reaction mixture was warmed to the reflux temperature. Thionyl chloride was added over a period of twenty minutes, after which the reaction mixture clarified to form a light amber-colored solution. Refluxing was continued for an additional hour. The chloroform and excess thionyl chloride were removed by distillation under reduced pressure. Two 20-cc. portions of absolute ethanol were added successively to the residue and then removed likewise. The light yellow-colored residue was basified with aqueous potassium hydroxide and solid potassium carbonate. The basic solution was then extracted with ether (four 25-cc. portions) and chloroform (four 25-cc. portions), the combined extracts dried over anhydrous sodium sulfate and Drierite, and the ether and chloroform removed from the dried filtered extract by distillation. Fractionation of the residue under reduced pressure yielded two fractions, 107–109°/12 mm. (2.8 Gm.) and 129–130°/12 mm. (2.3 Gm.). The second fraction was identified as 2-hydroxyoctahydroquinolizine by a mixed melting point determination. The methobromide salt of the first fraction was prepared in the usual manner, crystallized three times from isopropyl alcohol to yield white needles, m. p. 238–239°, dried and submitted for analysis.

Anal.—Calcd. for $C_{10}H_{18}NClBr$: C, 44.8; H, 7.2. Found: C, 44.8; H, 7.4.

Preparation of the Cycloaliphatic Acids

Cyclohexylacetic Acid.—A suspension of phenylacetic acid (6.0 Gm.), previously purified by two recrystallizations from an acetic acid-water mixture and melting at 77–78° (10), and platinum oxide catalyst (0.2 Gm.) in glacial acetic acid (30 cc.) was hydrogenated under an initial pressure of 60 p. s. i. until the calculated amount of hydrogen had been absorbed (2 hr.). After filtration and removal of the solvent by distillation under reduced pressure, the remaining viscous residue was fractionated under reduced pressure. The fraction distilling at 111°/5 mm. was collected (5.7 Gm., 91% of theory). The literature records 110–112°/3 mm. (10) for cyclohexylacetic acid.

Dicyclohexylacetic Acid.—Diphenylacetic acid was purified (m. p. 148–9°; lit. m. p. 148° (11)) and hydrogenated in the manner described above. The product was crystallized twice from a dioxane-water mixture, m. p. 138–139° (lit. m. p. 137° (12) and 138.6° (13)). The yield was 69.2% of theory.

Hexahydrobenzylacetic Acid.—Benzylacetic acid[6] was purified (m. p. 50–51°; lit. m. p. 48.6° (11)) and hydrogenated in the manner previously described. The product distilled at 138°/9 mm. (lit. 112–114°/4 mm. (10)) and was obtained in 84% yield.

Dihexahydrobenzylacetic Acid.—This acid was obtained in 80% yield by hydrogenation of purified dibenzylacetic acid in the same way as cyclohexylacetic acid. The product was crystallized twice from a mixture of acetone and water to give colorless rhombic platelets, m. p. 74–75°.

Representative Syntheses of Octahydroquinolizin-2-yl Esters

Octahydroquinolizin - 2 - yl Diphenylacetate.—Thionyl chloride (10 Gm.) was added to diphenylacetic acid (2.74 Gm., 0.0129 mole) contained in a 250-cc. round-bottom flask equipped with a reflux condenser and protected from moisture with a calcium chloride drying tube. After the mixture had been refluxed for two hours, the excess thionyl chloride was removed by distillation under reduced pressure. The greenish yellow-colored residue was dissolved in anhydrous benzene (25 cc.) and a solution of 2-hydroxyoctahydroquinolizine (2.00 Gm., 0.0129 mole) in anhydrous benzene was added to it. The reaction mixture was refluxed on a steam bath for nineteen hours and then chilled in an ice bath. Ethereal hydrogen chloride was then added in excess to the chilled reaction mixture and the benzene and

[6] Although the correct name for this compound is 2-phenylpropionic acid, all the acids employed in the esterification of 2-hydroxyoctahydroquinolizine, except for fluorene-9-carboxylic and xanthene-9-carboxylic acids, have been named as substituted acetic acids in order to emphasize structural analogies.

ether removed by distillation under reduced pressure. The residue was washed with anhydrous petroleum ether (five 30-cc. portions) and then transferred to a desiccator containing phosphorus pentoxide. After standing in the evacuated desiccator for twenty-four hours, the residue weighed 4.10 Gm.

Assuming the residue to consist entirely of ester hydrochloride and unreacted amino alcohol hydrochloride, the following calculations were employed to determine the extent to which the amino alcohol had been esterified:

Let x = moles of amino ester hydrochloride
$(0.0129 - x)$ = moles of amino alcohol hydrochloride
$385.92x$ = grams of amino ester hydrochloride
$191.70\ (0.0129 - x)$ = grams of amino alcohol hydrochloride
$385.92x + 191.70\ (0.0129 - x) = 4.10$ Gm.
$x = 0.0083$ mole of amino ester hydrochloride
$0.0083/0.0129\ (100) = 64.34\%$ of theory

Two grams of the total residue was dissolved in water and basified with aqueous potassium hydroxide. The basic solution was extracted several times with ether (five 30-cc. portions) and the combined extracts dried over anhydrous sodium sulfate and Drierite. After the ether had been removed from the dried extract by distillation, the residue was dissolved in petroleum ether (20 cc.) and chromatographed through an alumina column (128 x 28 mm.; 75 Gm. aluminum oxide, reagent grade, for chromatographic purposes, Merck) using petroleum ether as the developer and petroleum-ether-benzene mixtures (increasing benzene content) as the eluant. Each fraction (20 cc.) collected was tested with Valser's reagent at the middle and end of its collection. Fractions 1–5 were negative to Valser's reagent, 6–25 were positive, 26–29 were negative, 30–41 were positive, and 42 on were negative. In each case, treatment of the combined fractions, 6–25 and 30–41, with ethereal hydrogen chloride yielded a white precipitate. The precipitates were collected, washed several times with anhydrous ether, dried and weighed:

Fractions	Weight, Gm.	% of "2 Gm."
6–25	1.27	63.5
30–41	0.65	32.5

The precipitate from fractions 30–41 crystallized from isopropyl alcohol-ether in the form of white needles, m. p. 259–260°. A mixed melting point determination of this compound with a sample of 2-hydroxyoctahydroquinolizine hydrochloride gave no depression of melting point. The precipitate from fractions 6–25 crystallized from acetone-petroleum ether in the form of white needles, m. p. 210–211°. Further data on this ester will be found in Table I. The remainder of the crude precipitate (2.10 Gm.) was treated in a similar manner and it was noteworthy that the amino ester fraction, when converted to the methobromide, accounted for 61.2% of the sample chromatographed.

Octahydroquinolizin-2-yl Hexahydrobenzylacetate.—Hexahydrobenzylacetyl chloride was prepared from hexahydrobenzylacetic acid (2.27 Gm., 0.013 mole) and thionyl chloride (20 Gm.). After the excess thionyl chloride had been removed in the usual manner, the acid chloride was dissolved in anhydrous benzene (60 cc.) and added to a solution of 2-hydroxyoctahydroquinolizine (4.00 Gm., 0.026 mole) in benzene (90 cc.). A white precipitate formed immediately and increased in quantity during the reflux period (5 hr.). After the benzene had been removed by distillation, the residue was basified with aqueous potassium hydroxide and extracted several times with petroleum ether (ten 30-cc. portions). The extracts were kept separate, and beginning with the first one, were extracted successively with the same 10-cc. portion of water. A total of ten 10-cc. portions of water was used to extract the petroleum ether extracts. The petroleum ether extracts were combined and dried with anhydrous sodium sulfate and Drierite. The dried extract was then filtered, the petroleum ether removed by distillation, and the residue dissolved in anhydrous ether (50 cc.). The ethereal solution was divided into two approximately equal portions; ethereal hydrogen chloride was added to one portion and ethereal methyl bromide to the other. The amino ester hydrochloride crystallized from acetone-petroleum ether in the form of colorless platelets (2 Gm.), m. p. 180–181.5°. The amino ester methobromide crystallized from a mixture of methyl isobutyl ketone and absolute ethanol in the form of white needles (1.8 Gm.), m. p. 205–206.5°. The yield of octahydroquinolizin-2-yl hexahydrobenzylacetate was 84.7% of theory.

Octahydroquinolizin - 2 - yl Phenylacetate.—Phenylacetyl chloride, prepared from phenylacetic acid (2.47 Gm., 0.016 mole) and thionyl chloride (20 Gm.), was dissolved in anhydrous benzene (60 cc.) and added to a solution of 2-hydroxyoctahydroquinolizine (4.96 Gm., 0.032 mole) in anhydrous benzene (80 cc.). The reaction mixture was refluxed for five hours and then cooled in an ice bath. Ethereal hydrogen chloride was added to the chilled reaction mixture and the benzene and ether subsequently removed by distillation under reduced pressure. The residue was washed several times with anhydrous petroleum ether (six 30-cc. portions), air dried, and transferred to a desiccator charged with phosphorus pentoxide. After standing twenty-four hours in the evacuated desiccator, the residue weighed 7.85 Gm. and indicated, by calculation, the presence of 4.48 Gm. of the amino ester hydrochloride (90.7% of theory).

The residue was dissolved in water and basified with aqueous potassium hydroxide and then extracted with ether (six 30-cc. portions). The ethereal extracts were dried in the usual way and the filtered and dried extract was then treated with an equivalent quantity of ethereal hydrogen chloride. The white precipitate was dissolved in hot acetone and the solution was then allowed to cool undisturbed in the presence of an added crystal of 2-hydroxyoctahydroquinolizine hydrochloride. Slow spontaneous evaporation of the acetone yielded a crop of needles, m. p. 259–260°. The mother liquor, under the same conditions, yielded two additional crops, m. p. 248–252° and 174–177°, respectively. The higher melting hydrochloride was identical with 2-hydroxyoctahydroquinolizine hydrochloride. The fourth, fifth, and sixth crops crystallized as colorless platelets, m. p. 159–162°, 161–163°, and 166–167°, respectively. The fourth and fifth crops were combined and recrystallized

from acetone, using a crystal from the sixth crop for a seed. This solution yielded colorless platelets, m. p. 166–167°. The weight of the combined recrystallized fourth, fifth, and sixth crops was 3.28 Gm. (66.21% of theory). Further data will be found in Table I.

Attempted Synthesis of Octahydroquinolizin-2-yl Xanthene-9-carboxylate

Reaction of Xanthene-9-carboxylic Acid with 2-Chloro-octahydroquinolizine (14, 15).—The reaction of xanthene-9-carboxylic acid and 2-chloro-octahydroquinolizine in isopropyl alcohol was carried out according to the general procedures used by Horenstein and Pählicke (15) as well as Burtner and Cusic (14). The reaction failed to yield a product and most of the acid was recovered unchanged.

Reaction of Silver Xanthene-9-carboxylate with 2-Chloro-octahydroquinolizine.—Xanthene-9-carboxylic acid was converted to the silver salt by first converting it to the ammonium salt with concentrated ammonia and then treating the solution with an equivalent amount of silver nitrate upon which the silver salt precipitated as a white precipitate. When silver xanthene-9-carboxylate was treated with an equivalent amount of 2-chloro-octahydroquinolizine in anhydrous xylene, no esterification was noted but instead a portion of the acid was found to have been converted to xanthone and the 2-chloro-octahydroquinolizine was recovered unchanged.

Pharmacological Data

The ganglionic blocking activity (standard, tetraethylammonium bromide = 1) was measured by the decrease in height of nictitating membrane contraction by blockade of transmission of electrical stimulus through the superior cervical (sympathetic) ganglion. The anticholinergic, neuroeffector blocking activity (standard, atropine sulfate = 1) was measured by the decrease in height of spastic contraction of the isolated rabbit ileum due to acetylcholine.

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Some Properties and Uses of Nu Film Starch*

By M. H. ALLEN, A. C. GRAVEL, and F. P. COSGROVE

The effects of concentration, pasting temperature, *p*H, and electrolytes on the viscosity and clarity of Nu Film starch gel are reported. The suspending and emulsifying properties of cornstarch and Nu Film starch are compared using several drugs and oils. The results indicate that Nu Film starch is generally superior to cornstarch as a suspending and emulsifying agent.

THIS STUDY was undertaken to determine some physical properties and uses of Nu Film starch[1] which might be of pharmaceutical interest. While considerable work has been reported on the properties of various natural starches (1), little experimental work has been published on derived starches which contain different polyelectrolyte groups.

Nu Film starch is the sodium salt of an ungelatinized, low-substituted starch derivative containing carboxylic and sulfonic acid groups. The replacement of some of the original hydroxyl groups of the starch with the acid groups, as sodium salts, has resulted in a molecule which contains a balanced combination of the three polar groups (1). The new product is commercially available as a finely divided free flowing white powder which has a high water-absorbing capacity and a low gelatinization temperature.

* Received April 13, 1956, from the College of Pharmacy, Loyola University, New Orleans, La.
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[1] Supplied by National Starch Products, Inc., Chicago, Ill.

EXPERIMENTAL

This starch, like many natural starches, when suspended in an aqueous mixture must be protected against bacterial and mold growth. Most commercial preservatives are suitable for this purpose (1). In the stabilizing, suspending, and emulsifying studies of this report 0.15% methyl *p*-hydroxybenzoate and 0.05% propyl *p*-hydroxybenzoate were employed. No preservatives were used in the

TABLE I.—THE EFFECT OF CONCENTRATION AND PASTING TEMPERATURE UPON THE VISCOSITY AND CLARITY OF TEST SAMPLES

Percentage of Starch[a]	Pasting Temperature	Viscosity in Centipoises[b] Range	Viscosity in Centipoises[b] Average of Five Readings	Transmittance
3	80	1664–1728	1680	60
..	85	1920–2560	2320	64
..	90	2240–2640	2480	65
..	95	2144–2480	2320	66
..	99.5	2000–2304	2176	68
6	80	4320–5024	4400	35
..	85	4448–4576	4480	40
..	90	5040–6400	5820	40
..	95	5360–5664	5520	45
..	99.5	4880–6000	5360	47
8	80	6240–6600	6400	..[c]
..	90	6560–8280	7440	22
..	99.5	6720–8160	7520	22

[a] Dry weight.
[b] Brookfield Helipath Stand Attachments measured at 25°: spindle TB, speed 50 r. p. m. for the 3% and 6% preparations; spindle TA, speed 20 r. p. m. for the 8% preparation pasted at 80 and 90°; spindle TC, speed 50 r. p. m. for the 8% preparation pasted at 99.5°.
[c] Too opaque to measure.

viscosity and clarity experiments since it was felt such agents might interfere with the final results.

Preparation of Test Samples.—The results of preliminary experiments indicated that the mode of preparation had little influence on the viscosity and clarity of our samples. The following slurry method was used chiefly because it required less time to perform:

Suspend the starch in cold distilled water (approximately 12% of the total volume), add to the remainder of water previously heated to the desired temperature, and mix well. Maintain the mixture at the prescribed temperature with constant stirring for one minute and then allow the mixture to cool slowly to 25°.

The Effect of Concentration and Pasting Temperature.—It has been previously noted (2–4) that the viscosities of aqueous preparations of many suspending agents such as Irish moss and potato starch were not consistent over a wide range of concentrations. Whittenberger and Nutting (4) reported that uniform gels of 10% or greater potato starch concentrations were prepared with difficulty. These workers also noted that the strength of potato starch gel depended largely upon the temperature at which the gel was heated. Raising the temperature from 85 to 95° decreased the gel strength by half, irrespective of the concentration. Woodruff and Nicoli (5) indicated that heating the potato starch gel to 90, 95, and 99.5° gave indistinguishable results; however, they made no quantitative measurements of the gel properties.

In order to determine the effect of concentration and pasting temperature upon the viscosity and translucency of aqueous preparations of Nu Film starch, concentrations of 3%, 6%, and 8% were prepared as previously described at various temperatures from 80 to 99.5°.

Viscosity measurements were made with a Brookfield Synchro-Lectric Viscometer-Helipath Stand attachment,[2] while clarity readings were obtained with the Coleman Universal Spectrophotometer, Model 14.[3] Results appear in Table I.

[2] Brookfield Engineering Laboratories, Stoughton, Mass.
[3] Coleman Instruments, Inc., Maywood, Ill.

Effect of pH.—Since the test starch is the sodium carboxylate and sodium sulfonate derivative of starch, it was of interest to determine the stability of this starch over a range of *p*H values. Whittenberger and Nutting (4) found that *p*H values from 4 to 9 had little effect upon potato starch gel but at *p*H values below 4 and above 9 there was considerable decrease in gel strength. They also found that the translucency of the gels was influenced by *p*H. Maximum translucency was exhibited between *p*H 6.5 and 7.5. Below *p*H 6 and above *p*H 8 translucency of the gels decreased.

To test the effect of *p*H upon the viscosity and clarity of our samples, 5% preparations were made at *p*H values from 2.3 to 8.3 and tested as previously described. Results are given in Table II.

TABLE II.—THE EFFECT OF *p*H ON VISCOSITY AND CLARITY OF 5% SAMPLES[a]

Pasted at *p*H Values[b]	Viscosity in Centipoises[c] Range	Viscosity in Centipoises[c] Average of Five Readings	Transmittance
2.3	2240–2800	2552	39
3.3	3200–4100	3696	46
4.3	3440–4080	3696	49
5.3	4000–4160	3904	49
6.3[d]	3840–4320	4010	48
7.3	3600–3840	3776	48
8.3	3580–3840	3680	48

[a] Pasting at 99.5° dry weight.
[b] Adjusted with HCl or NaOH.
[c] Brookfield Helipath Stand Attachment measured at 25°; spindle TB, speed 50 r. p. m.
[d] *p*H of distilled water.

Effect of Electrolytes.—Electrolytes in adequate concentrations are generally incompatible with colloidal solutions for they compete with the colloids for the water, causing dehydration and a decrease in the viscosity of the preparations. Since sulfonic acid and carboxylic acid groups, as the sodium salts, are present in the molecule of the test starch, electrolytes may also combine with the negative hydrophilic residue to form other salts that

are less soluble. Because of these possibilities it was desirable to study the effect of sodium chloride, calcium chloride, and aluminum chloride (0.01 *N*) upon the viscosity and translucency of 6% pastes on Nu Film starch and cornstarch. Results appear in Table III.

sol-like, while the 5%, 6%, and 8% samples were fairly thick pastes or gel-like products.

With trial experiments it was observed that some gelatinization of the starch occurred at 60°. This temperature is approximately 15°

TABLE III.—THE EFFECT OF ELECTROLYTES UPON THE VISCOSITY AND CLARITY OF 6% SAMPLES[a]

Starch	Type and Concentration of Electrolyte Solution	Viscosity in Centipoises[b] Range	Viscosity in Centipoises[b] Average of Five Readings	Transmittance
Nu Film	a. Distilled Water (Control)	4880–6000	5360	47
	b. Sodium Chloride (0.01 *N*)	3840–4096	3980	36.5
	c. Calcium Chloride (0.01 *N*)	2240–2560	2448	21.0
	d. Aluminum Chloride (0.01 *N*)	1312–1472	1392	..[c]
Cornstarch	a. Distilled Water (Control)	3264–3360	3296	..[c]
	b. Sodium Chloride (0.01 *N*)	3040–3200	3168	..[c]
	c. Calcium Chloride (0.01 *N*)	2400–2464	2032	..[c]
	d. Aluminum Chloride (0.01 *N*)	1712–1872	1811	..[c]

[a] Pasted at 99.5° dry weight.
[b] Brookfield Helipath Stand Attachments measured at 25°; spindle TB, speed 50 r. p. m. for Nu Film starch; spindle TA, speed 50 r. p. m. for cornstarch.
[c] Too opaque to measure.

Stabilizing, Suspending, and Emulsifying Studies.—It has been reported (1) that the test starch has very high water-absorbing properties which give it excellent stabilizing and suspending qualities in aqueous preparations. While our preliminary studies indicated that simple aqueous suspensions of the starch have little stabilizing and suspending properties, 1.5% and 3.0% preparations made by adding the starch to boiling water and then cooling the mixture to 25° possessed satisfactory stabilizing and suspending qualities for a number of solids and liquids. In this study thirteen formulations were used to evaluate the efficiency of the new stabilizer. Cornstarch was used as a control. Suspensions were made, in a mortar, by triturating the ingredients with a small amount of the suspending solution to form a smooth paste and then gradually diluting to volume with more of the suspending solution. The samples were placed in 100-ml. cylindrical graduates, set aside for twenty-four and one-hundred-twenty hours, and then examined for the percentage of settling. Emulsions were prepared either by placing the oil in a dry mortar and slowly adding the starch solution, with trituration, until the required volume was obtained or by adding the oil to the required amount of starch solution, mixing well, and then passing the emulsion through a hand homogenizer. Satisfactory preparations were obtained with both methods. Emulsions were placed in tightly stoppered glass containers and examined for general appearance after twenty-four and one-hundred-twenty hours.

Ingredients used, concentrations of formulations prepared, and results of the studies are listed in Tables IV and V.

DISCUSSION AND SUMMARY

The physical appearance of the test samples varied with the concentration of the starch. Three per cent preparations were thin pastes or lower than the initial gelatinizating temperature of cornstarch.

More satisfactory viscosity readings were obtained with the Helipath Stand Attachment than with the Standard Brookfield Viscometer, because throughout the helical path of the Helipath crossbar spindle or rotor new material, with undisturbed structure, was constantly encountered. However, it has been reported (6) that no absolute correlation of results can be made using different crossbars or using the same crossbar at different speeds.

Clarity of samples expressed as percentage transmittance compared with water was determined according to the method of Whittenberger and Nutting (4) using the Coleman Universal Spectrophotometer, Model 14, with Red Filter No. P.C. 5 at 750.0 mμ. With this long wavelength it was possible to measure the transmittance of samples of relatively high opacity and also to avoid interferences due to impurities absorbing at shorter wavelengths.

Raising the pasting temperature from 80 to 90° gradually increased the viscosity of the 3% and 6% preparations; however, there was an overlapping of readings at some temperatures. Additional pasting temperatures lowered the viscosity of these preparations. Clarity of the test samples generally increased with the pasting temperature, although with some samples little or no differences were observed. Uniform samples of 8% Nu Film starch were difficult to prepare, and the data on these preparations show greater fluctuations than those on the less concentrated samples. Such variations may be

TABLE IV.—STABILIZING AND SUSPENDING PROPERTIES OF NU FILM STARCH AND CORNSTARCH

Stabilizer	Cornstarch				Nu Film Starch			
Percentage Stabilizer	1.5%	1.5%	3.0%	3.0%	1.5%	1.5%	3.0%	3.0%
Reading Times in Hours	24	120	24	120	24	120	24	120
Solids Suspended								
Bismuth Subnitrate 10.0% Dist. water *q. s.*[a]	31% settling	51% settling[b]	No settling[b]	No settling[b]	...[d]	...[d]	...[d]	...[d]
Sulfathiazole 10.0% Dist. water *q. s.*[a]	40% settling	45% settling	No settling[b]	4% settling[b]	No settling	5% settling	No settling	1% settling
Salicylic Acid 3.0% Ammoniated Mercury 3.0% Dist. water *q. s.*[a]	22% settling	37% settling	1% settling[b]	6% settling[b]	5% settling	50% settling	No settling	4% settling
Pptd. Sulfur 10.0% Dist. water *q. s.*[a]	30% settling	50% settling	No settling[c]	10% settling[c]	20% settling	25% settling	No settling	5% settling
Calamine 8.0% Zinc Oxide 8.0% P.E.G. 400 8.0% Dist. water *q. s.*[a]	50% settling	60% settling	10% settling[b]	26% settling[b]	70% settling	74% settling	4% settling	6% settling
Zinc Oxide 10.0% P.E.G. 400 5.0% Dist. water *q. s.*[a]	50% settling	53% settling[b]	5% settling	26% settling	72% settling	48% settling	4% settling	8% settling
Calamine 8.0% Dist. water *q. s.*[a]	40% settling	50% settling	No settling	No settling[c]	3% settling	4% settling	...	...
Zinc Oxide 10.0% Dist. water *q. s.*[a]	50% settling	53% settling	No settling	No settling[c]	5% settling	8% settling	...	...
Ichthammol 10.0% Dist. water *q. s.*[a]	No settling	No settling	No settling	No settling	No settling	No settling	No settling	No settling
Ichthammol 25% Dist. water *q. s.*[a]	No settling	No settling	No settling	No settling	No settling	No settling	No settling	No settling

[a] Starch added to the water previously heated to 99.5°.
[b] A thick gelatinous-like product formed. Upon shaking a sol-like product formed.
[c] A thick gelatinous-like product formed. Did not form a sol-like product upon shaking.
[d] There was a chemical incompatibility.

TABLE V.—EMULSIFYING PROPERTIES OF NU FILM STARCH AND CORNSTARCH

Emulsifier	Cornstarch				Nu Film Starch			
Percentage Emulsifier	1.5%	1.5%	3.0%	3.0%	1.5%	1.5%	3.0%	3.0%
Reading Time in Hrs.	24	120	24	120	24	120	120	120
Liquids Suspended								
Cotton Seed Oil 25% Dist. water *q. s.*[a]	10% separation	20% separation	10% separation	20% separation	No separation	No separation	No separation	No separation
Cotton Seed Oil 50% Dist. water *q. s.*[a]	10% separation	25% separation	2% separation[c]	12% separation[c]	3% separation	26% separation	No separation	No separation
Cotton Seed Oil 75% Dist. water *q. s.*[a]	Thick mass	Thick mass	Thick mass	Thick mass	17% separation	30% separation	70% separation	85% separation
Mineral Oil 25% Dist. water *q. s.*[a]	40% separation	53% separation	30% separation	61% separation	1% separation	2% separation	No separation	No separation
Mineral Oil 50% Dist. water *q. s.*[a]	2% separation[c]	5% separation[c]	1% separation[c]	3% separation[c]	4.5% separation	32% separation	No separation	2% separation
Mineral Oil 75% Dist. water *q. s.*[a]	Cracked	Cracked	80% separation	92% separation	85% separation[d]	95% separation[d]	65% separation[d]	90% separation[d]
Castor Oil 25% Dist. water *q. s.*[a]	No separation[c]	No separation[c]	No separation[c]	No separation[c]	No separation[b]	No separation[b]	No separation[b]	No separation[b]
Castor Oil 50% Dist. water q.s.[a]	15% separation	21% separation	No separation[c]	No separation[c]	5% separation[b]	5% separation[b]	No separation[b]	No separation[b]
Castor Oil 75% Dist. water *q. s.*[a]	86% separation[d]	98% separation[d]	No separation[c]	No separation[c]	70% separation[c]	85% separation[c]	80% separation[c]	82% separation[c]
Turpentine Oil 25% Dist. water *q. s.*[a]	60% separation	85% separation	45% separation	65% separation	8% separation	10% separation	No separation	No separation
Turpentine oil 50% Dist. water *q. s.*[a]	80% separation[d]	91% separation[d]	8% separation[c]	20% separation[c]	5% separation	5% separation	No separation	No separation
Turpentine oil 75% Dist. water *q. s.*[a]	85% separation	92% separation	70% separation	82% separation	75% separation	90% separation	70% separation	90% separation

[a] Starch added to the water previously heated to 99.5°.
[b] Too thick to pour.
[c] A thick gel-like mixture formed on standing.
[d] Dispersion of globules of oil throughout the mixture resulting in a thick mass.

due, in part, to the increased concentration of starch, to the incomplete pasting of the starch, or to the heterogeneity of the samples. It is felt that preparations containing 8% or higher Nu Film starch concentration deserve further study.

With samples prepared at 99.5°, the effect upon viscosity of varying the *p*H between 3.3 and 8.3 was slight but at *p*H 2.3 there was a considerable decrease in viscosity over the control prepared with distilled water (*p*H 6.3). Translucency of the samples was influenced slightly by *p*H. At *p*H values of 3.3 and 2.3 there was a decrease in clarity over the control but from *p*H 4.3 to *p*H 8.3 the readings were almost identical.

Both the viscosity and clarity of samples decreased with the addition of electrolytes.

Results of our study indicate that Nu Film starch is a valuable agent for preparing some emulsions and suspensions. In general, 3% starch was required to stabilize the formulations studied, and in most cases this concentration exhibited greater stabilizing properties than 3% cornstarch. Satisfactory suspensions were obtained with sulfur, salicylic acid and ammoniated mercury, calamine and zinc oxide formulations. With salicylic acid and ammoniated mercury, however, a distinct chemical incompatibility was observed with samples stored for thirty days at 25°. Excellent suspensions were obtained with sulfathiazole and ichthammol. Bismuth subnitrate was definitely incompatible with the suspending agent. Fifty per cent concentrations of cottonseed oil, mineral oil, castor oil, and turpentine oil were satisfactorily emulsified with a 3% preparation of the test starch. However, with castor oil, the emulsions were too thick to pour. Three per cent starch preparations gave unsatisfactory results with oil concentrations of 75%.

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Sorption of Water by Rubber Closures for Injections

I. Effect of Inorganic Salts*

By GEORGE MILOSOVICH† and ALBERT M. MATTOCKS

In a search for a physicochemical method for predicting stability of rubber closures with injection solutions the sorption characteristics of a representative group of rubber compositions have been studied. This paper reports the effects of twenty inorganic compounds on the sorption of water by the closures. The general effect of inorganic ions was shown to be due to the lowering of relative vapor pressure of the solution, resulting in decreased sorption. Bisulfite ion was found to be an exception to the rule and caused marked increase in sorption. This was explained on the basis of penetration of the rubber by bisulfite or a derivative thereof, rendering the rubber more hydrophyllic. The effect of lowering of the relative vapor pressure upon sorption for the closures tested was found to be dependent upon the percentage rubber in the composition.

RUBBER has been the most satisfactory material found to date for use as closures for multidose injection vials. In addition to being self-sealing after puncture, it adheres to the glass of the vial making a good seal against bacterial entry and is relatively stable to autoclaving. In spite of these advantages it causes many difficulties in this use. Chemical and physical reactions frequently occur between the parenteral solutions and the rubber to make the product useless. Such changes are seen as discoloration or swelling of the rubber, loss of

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potency of the medicinal, or cloudiness of the solution.

It is common practice in industry to run stability tests on various rubber closures and injection formulations to determine which combination will result in a compatible or stable product. Such tests usually consist of observing the closure and solution after they have been in contact with each other for two months at 45°. If no visible signs of reaction are present and no loss of material from solution occurs in a closure-solution combination it is considered to be stable.

The drawbacks of this type test are quite evident. In addition to being time consuming, the test cannot predict long range stability with any great degree of accuracy since accelerated tests of this nature are not representative of actual storage conditions. As a result there is a definite need in the pharmaceutical industry for a short but conclusive stability test for rubber closures which may also be used as a means of quality control for closures.

In a symposium on containers and rubber closures, Barry (1) sets forth four criteria for judging rubber closures; (*a*) physical characteristics, (*b*) yielding of extractives to the solution, (*c*) sorption of substances by rubber from solutions, (*d*) effects of rubber on medicaments. Since physical characteristics are "built" into rubber closures by compounding and most of the closures offered today possess the desirable physical properties, we are concerned only with obtaining a means for determining (*b*), (*c*), and (*d*).

A literature search revealed that most of the work done on this problem has been directed toward developing chemical tests for materials extracted from the rubber. Christiansen (2) and Steiger and Dolder (3) have described various chemical tests used to determine stability, and the latter concluded that since every closure tested gave positive chemical tests, prediction of stability could not be made on this basis. Reznek (4) measured the amount of zinc leached from closures by solutions and found it to vary over wide limits between different lots of the same closure. Again no positive conclusions were drawn which would indicate that stability could be predicted from this information.

Rubber closures in use at present are very complex in chemical nature. In addition to the inorganic and organic compounds present in natural rubber many more materials are added to modify the physical properties of the rubber composition. For example, of six of the most popular formulas of one manufacturer only one has less than seven additives and one has as many as ten.

Many of these substances present in rubber are thought of as highly reactive compounds, and the number of possibilities for reaction with chemicals which might be present in an injection is so large as to discourage chemical investigation. Certainly a chemical investigation of a single combination of closure with a specific injection solution may be feasible, but no broad generalizations regarding any of the unlimited number of other combinations could be made. Because of this, it was felt that some physical test which would predict the likelihood of an injection solution coming into contact with the reactive ingredients of a rubber closure would best serve to predict stability of closure-solution combinations. If the surface of a rubber closure acts as an effective barrier between the substances contained within the rubber and the solution exterior to it then it would seem that chemical stability would result regardless of chemical reactivity of the rubber closure composition. On the other hand, if the rubber composition is penetrated by the solution, the reactivity of the chemicals within a rubber closure would almost assure chemical reaction.

Accordingly, physical tests which might be used to predict the effectiveness of the closure surface to act as a barrier were focused upon, and the first such investigation, reported in this paper, is a study of the sorption of water from various inorganic solutions by the closures.

In early papers Boggs and Blake (5) and Lowry and Kohman (6) report investigations into the mechanism of the sorption of water by rubber used for electrical insulation. Taylor and Kemp (7) found that the sorption process could be described by an equation of the form $w = kt^n$ where w is the weight of water sorbed in time, t, and n and k are constants dependent upon temperature, composition, physical properties of rubber, and the vapor pressure of the solution in contact with the rubber. Lowry and Kohman also give evidence that leaching of materials from rubber will be shown by a break in the continuity of the sorption curve due to its effect on the vapor pressure of the external solution.

The most acceptable theory for the sorption of water is that proposed by Lowry and Kohman (6). They postulate that the water which entered the rubber forms a saturated or concentrated solution of soluble ingredients within the rubber which has, consequently, a vapor pressure lower than that of the water on the exterior. This difference in vapor pressure is the driving

force of sorption, the water tending to enter the rubber to equalize the vapor pressures within and without. Sorption would continue *ad infinitum* were it not for the resistance of the rubber to stretching. Long periods of time are required before the stretching effect manifests itself by retarding the sorption rate. If soluble material within the rubber is leached into the external solution, this would, of course, lower the outside vapor pressure and at the same time raise the inside vapor pressure, thus lowering the sorption rate. With water alone the internal vapor pressure can never equal that of the water unless leaching occurs, but with solutions it is possible to reach equilibrium in a finite time and in such case have sorption reach a maximum. The time required to attain the maximum would vary with type and quantity of soluble ingredients within the rubber as well as with the vapor pressure of the external solution. Kemp (8), for example, found sorption maxima for raw rubber immersed in 3.5% sodium chloride solutions to occur at sixty to eighty days.

According to Wentworth (9) sorption of water is one of the tests now used in ascertaining the value of crude rubber, and he presents a study of the methods used to determine it. The methods involve either measurement of weight gain of a rubber sample when it is placed in contact with water, or when in contact with water vapor. Identical results have been obtained by the two methods if no leaching occurs; however, since injection closures come in direct contact with solutions and not vapor alone it was felt that the immersion method would yield more representative results.

EXPERIMENTAL

Although there are many different closure compositions in common use, the present study was restricted to closures used with aqueous solutions. Of this group many were of quite similar composition, varying only in color or percentage of filler, so that it was possible to select six rubber compositions which were distinctly different and which represent practically every basic formula used with aqueous solutions. These representative types were West Company compositions called Gum 79, Pink 124, Red 87, White 86, Black 210, and Red 123. For convenience the small sized closures, 13-mm. diameter, were selected for the tests. The closures were not washed but taken directly as received from the manufacturer and dried to constant weight over sulfuric acid in vacuum. Silica gel in vacuum gave identical drying, and has been used subsequently as a drying method.

The sorption of water by the six closures was determined from twenty solutions of inorganic salts selected as representing the types of compounds used in injections. The solutions for this test were 0.5 *M*.

The sorption experiments were carried out in a constant temperature water bath at 30.1 ± 0.05°. This temperature was chosen because it was felt it should be as near room temperature as possible and yet be held constant throughout the experiment.

Reagent or pharmaceutical grade chemicals were used throughout and solutions were made using fresh triple-distilled water.

PROCEDURE

Water Sorption.—Three closures of a given type, previously dried to constant weight, were individually weighed and placed in a five-dram plastic screw cap glass vial. The solution to be tested was then added up to the neck of the vial and the vial closed. Several sharp taps of the vial served to remove air bubbles which adhered to the surface of the closures and the vial was placed in the water bath. At given intervals of time the closures were taken from the vial, the moisture adhering to their surface was removed with a blast of compressed air, and the stoppers were weighed. They were then replaced in the vial, fresh solution was added, and the vials were reimmersed as before. The increase in weight of the closures was assumed to be due to sorption of water.

RESULTS

In the initial sorption experiments reproducibility between the individual closures selected for a test was not as good as expected in some instances. In an attempt to determine the cause for variation the sorption of water was compared with density measurements on individual closures of one type. Gum 79 was selected for this study since it showed the greatest individual variation. Density was measured by comparing the weight in air with loss of weight in water.

Densities and water sorption values of this group of individual closures are shown in Table I. Graphing these values showed a linear dependence of sorption upon density, and calculation gave a correlation coefficient of 0.90. This may be interpreted to indicate that 90% of the variation between individual stoppers is due to density differences. Since these variations were not due to errors of measurement but were true differences in individual closures, it was decided to include them in the data. Density also varies between different types of closures but since they were all approximately the same volume and the same shape their surface

TABLE I.—RELATIONSHIP OF WATER SORPTION TO DENSITY AND VOLUME OF GUM 79 CLOSURES

Closure	Density, Gm./cc.	Volume, cc.	Sorption, Gm.
1	0.9820	1.2967	0.0085
2	0.9154	1.2765	0.0056
3	0.9165	1.2760	0.0063
4	0.9265	1.2751	0.0053
5	0.9773	1.2714	0.0081
6	0.9335	1.2745	0.0065
7	0.9182	1.2756	0.0053
8	0.9042	1.2759	0.0050

TABLE II.—SORPTION CONSTANTS FOR RUBBER CLOSURES IN INORGANIC SOLUTIONS

$$\log w = n \log t + \log k$$

Closure Type → / Solution	Gum 79		Pink 124		Red 123		Black 210		White 86		Red 87	
	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$
H_2O	0.390	0.454	0.388	0.452	0.426	0.459	0.370	0.491	0.448	0.355	0.441	0.397
Na_2SO_4	0.317	0.416	0.304	0.420	0.330	0.317	0.211	0.477	0.321	0.269	0.315	0.300
NaCl	0.327	0.387	0.293	0.447	0.348	0.294	0.242	0.415	0.323	0.292	0.326	0.269
Na_3PO_4	0.356	0.002	0.281	0.285	0.334	0.192	0.132	0.357	0.309	0.128	0.366	0.107
$NaHSO_3$	...	...	...	...	...	...	...	...	0.812	0.531	0.841	0.696
$Na_2S_2O_3$	0.376	0.354	0.322	0.407	0.372	0.290	0.250	0.415	0.355	0.226	0.362	0.261
$NaNO_3$	0.373	0.370	0.319	0.403	0.367	0.280	0.224	0.441	0.338	0.263	0.343	0.268
NaBr	0.385	0.321	0.358	0.325	0.363	0.279	0.225	0.389	0.311	0.273	0.344	0.269
$MgSO_4$	0.372	0.401	0.344	0.488	0.416	0.329	0.270	0.503	0.389	0.297	0.372	0.334
$Mg(NO_3)_2$	0.336	0.357	0.239	0.487	0.353	0.258	0.190	0.399	0.347	0.229	0.305	0.242
$MgBr_2$	0.303	0.388	0.255	0.460	0.359	0.253	0.183	0.408	0.340	0.238	0.305	0.263
$MgCl_2$	0.353	0.369	0.280	0.449	0.365	0.266	0.215	0.386	0.376	0.198	0.323	0.263
$CaBr_2$	0.291	0.437	0.258	0.549	0.308	0.363	0.190	0.459	0.292	0.291	0.257	0.338
$Ca(NO_3)_2$	0.309	0.395	0.230	0.478	0.294	0.330	0.157	0.429	0.305	0.251	0.281	0.308
$CaCl_2$	0.304	0.401	0.257	0.479	0.300	0.323	0.183	0.417	0.301	0.281	0.266	0.281
KCl	0.381	0.418	0.299	0.476	0.377	0.329	0.222	0.495	0.317	0.335	0.344	0.315
KBr	0.316	0.411	0.274	0.519	0.350	0.336	0.200	0.481	0.356	0.301	0.317	0.314
K_2SO_4	0.332	0.387	0.294	0.486	0.376	0.312	0.216	0.447	0.323	0.341	0.294	0.364
$KHSO_3$	0.460	0.586	0.574	1.152	0.610	0.908	0.707	0.762	0.675	0.776	0.730	0.958
KNO_3	0.332	0.429	0.280	0.528	0.354	0.333	0.224	0.468	0.349	0.285	0.299	0.336
K_3PO_4	0.390	0.036	0.229	0.462	0.426	0.292	0.120	0.397	0.346	0.157	0.349	0.112

areas are approximately equal. Therefore, a better comparison is obtained if results are recorded as mg. water sorbed per closure.

It should be pointed out that this individual variation due to differences in closure density is included in the error of the experiment and the only difficulty it introduces is a loss of sensitivity. That is, two solutions must show a larger difference in sorption for the effect to be significantly greater than the variations between individuals in the same solution. The sensitivity was still considered to be acceptable, even for closures having the greatest individual variation. For example, with Gum 79 and sodium sulfate solution a large deviation was encountered. However, the standard deviation of the points from the line (log w *vs.* log t) was calculated to be 0.0575 giving a confidence range (95% level) for n of ± 0.049, for log k of ±0.023, and for the one-year extrapolation value of ±0.0058 Gm. On the other hand, with Red 123, which had small individual variation, when treated with 2-butanone solution, the standard deviation was 0.0118 giving a confidence range (95% level) for n of ±0.009, for log k of ±0.005, and for the one-year extrapolation value of ±0.0021 Gm. It was considered unnecessary to calculate the individual errors for each combination investigated since it is obvious that they will fall between the two extremes cited above with the majority approaching the lower limit.

Since no comparison can be obtained directly from the constants n and k it was felt that an extrapolation value at some given time could be used for comparison purposes. The extrapolation value should represent some point on the line far enough out on the log time axis to avoid points of convergence of two curves with widely different slopes but which have different intercepts and might thus give misleading comparisons. It is recognized that extrapolation of the line may not represent values that would be obtained from experimental measurements, since it does not take into account changes which might occur in the system over a long period of time. Such changes as aging of the rubber (oxidation), leaching, and attainment of equilibrium would change the system, and the sorption curve would no longer be expected to follow the same equation. Extrapolated values of one year were thus arbitrarily selected as suitable for comparisons, and these values are reported in addition to the sorption constants.

Table II gives the constants obtained with inorganic solutions. The constants n and log k were calculated by the method of least squares from the logarithmic transformation of the equation $w = kt^n$; i. e., $\log w = n\log t + \log k$. For four of the combinations involving sodium bisulfite no values for these constants are given since a linear relationship was not obtained using the above equation. Table III presents the one-year extrapolation values calculated from the constants reported in Table II.

As seen from Table III, all the inorganic salts except bisulfite depressed the sorption of water by the closures. This would be predicted by the theory of Lowry and Kohman since the addition of a salt to water would decrease the relative vapor pressure. If it is assumed, as is suggested by the work of Kahlenberg (10) with sodium chloride and silver nitrate on rubber membranes, that inorganic salts do not diffuse through the rubber, then the decrease in vapor pressure due to the presence of the salt would be the main effect of these inorganic compounds. This appears to be true, since, first of all, every inorganic solution except bisulfite gave lower sorption with every stopper than was obtained from water alone. The quantitative relationship however, between depression of relative vapor pressure and depression of sorption has not been established as yet; so that one is not able to predict the depression of sorption with a reduction of relative vapor pressure.

Potassium and sodium salts can be compared as to their depressant effect on sorption of water by the six closures, because all combinations of potassium,

TABLE III.—ONE-YEAR EXTRAPOLATION VALUES
Gm. × 10^4 Sorbed by Individual Closures

Closure Type→ Solution	Gum 79	Pink 124	Red 123	Black 210	White 86	Red 87
Water	283	495	356		318	337
Na_2SO_4	169	158	145	97	123	128
NaCl	168	158	153	108	132	127
Na_3PO_4	82	101	112	50	84	111
$NaHSO_3$	...	...	...	...	4090	7100
$Na_2S_2O_3$	207	171	175	114	137	155
$NaNO_3$	212	166	166	103	135	140
NaBr	203	175	162	93	118	141
$MgSO_4$	226	234	248	157	197	194
$Mg(NO_3)_2$	165	126	145	77	131	106
$MgBr_2$	146	130	149	75	129	111
$MgCl_2$	187	147	159	87	145	123
$CaBr_2$	152	162	142	88	110	99
$Ca(NO_3)_2$	154	119	121	68	108	107
$CaCl_2$	151	138	124	77	113	92
KCl	248	175	197	146	140	157
KBr	166	166	171	98	163	134
K_2SO_4	173	175	188	100	148	131
$KHSO_3$	582	4200	2960	3750	3210	6730
KNO_3	191	176	174	110	151	127
K_3PO_4	92	112	126	51	111	101

sodium, chloride, nitrate, bromide, phosphate, and sulfate were tested. Magnesium and calcium salts could not be included in the same comparison since they could not be tested with all the same anions, though they can be compared with another group of anions. Values used for these comparisons were obtained by subtracting extrapolated values for each salt solution from the extrapolated values for water on the same closure.

The comparison of potassium and sodium salts is shown in Table IV, being made by means of a three-way analysis of variance It is seen from this table that anions have a general effect greater than any interactions in which they are involved, thus the different anions depress water sorption of closures to different degrees. Examination of mean effects of the individual anions by an lsd test shows phosphate ion to give significantly different effects from the other anions, but the effects of the other four are not shown to be different. If the effects of the anions are due simply to a lowering of relative vapor pressure this might be expected, for phosphate ion is known to give a much greater depression of vapor pressure than the other anions, whereas sulfate and bromide are practically equivalent in this effect and chloride and nitrate are only slightly different (11). This test was apparently not sensitive enough to detect the small differences that might be expected between chloride or nitrate and bromide or sulfate.

Also shown in Table IV is a significant interaction of cation with anion. This is interpreted as being an effect of individual inorganic chemical compounds beyond a simple additive effect of the constituent ions. This, too, can be explained on the basis of simple vapor pressure lowering, since the anion and cation species do affect one another in dissociation which in turn affects the vapor pressure of solution.

Although the cation effect shown in Table IV is significant compared to residual, the effect is shown to be not significantly greater than its interactions.

The calcium and magnesium salts were analyzed in the same manner, and the results are presented in Table V. The common anions were chloride, bromide, and nitrate. The analysis indicates that the anions are not different in their effect on sorption (anion effect) except as they are influenced by cations (anion × cation effect). Also, no difference between effects of magnesium and calcium ions could be detected. The interaction, cation × rubber, is significant because calcium had a greater

TABLE IV.—DEPRESSANT EFFECT OF SODIUM AND POTASSIUM SALTS ON SORPTION OF WATER BY SIX CLOSURE TYPES
Three-Way Analysis of Variance

Source	*s. s.*	*d. f.*	*m. s.*	*F*	*F/I*
Anion	33,088.66	4	8,272.2	42.5[a]	13.3[a]
Rubber composition	273,595.8	5	54,719.2	281.2[a]	...
Cation	2,331.33	1	2,331.33	12.0[a]	3.76
Anion × rubber	7,128.54	20	356.4	1.83	...
Anion × cation	2,482.61	4	620.7	3.19[a]	...
Cation × rubber	1,123.67	5	224.7	1.15	...
Residual	3,891.39	20	194.6	...	...
Total	...	59	...	...	...

[a] Significant at 5% or lower level.

TABLE V.—DEPRESSANT EFFECT OF CALCIUM AND MAGNESIUM SALTS ON SORPTION OF WATER BY SIX CLOSURE TYPES

Three-Way Analysis of Variance

Source	*s. s.*	*d. f.*	*m. s.*	*F*	*F/I*
Anion	564.23	2	282.11	5.61[a]	1
Rubber composition	177,542.1	5	35,508.4	705.6[a]	177.3[a]
Cation	1,260.25	1	1,260.25	25.0[a]	4.10
Anion × rubber	883.17	10	88.32	1.75	...
Anion × cation	1,148.6	2	574.3	11.41[a]	...
Cation × rubber	1,001.31	5	200.26	3.98[a]	...
Residual	503.34	10	50.33	...	...
Total	...	35	...	...	...

[a] Significant at the 5% or lower level.

depressant effect on water sorption of four closures, Red 87, Gum 79, Red 123, and White 86, but had an equal effect on Black 210 and a lesser effect on Pink 124.

The divalent ions, calcium and magnesium, would be expected to be different from the monovalent ones on the basis of vapor pressure reduction. Both types of cations had common anions: Chloride, bromide, and nitrate; so an analysis of the effects of these compounds was performed in the same manner as for Tables IV and V, and, as expected, the depressive effect was found to be significantly greater for the divalent cations.

In each of the analyses of variance, rubber compositions were shown to be different in their susceptibility to variations in relative vapor pressure caused by inorganic salts. The same order of susceptibility to this effect was shown in each analysis, this being the reverse order of the percentage rubber in the composition. That is, the closure type showing the greatest decrease in sorption had the lowest percentage rubber, etc. The order was as follows, with percentage rubber shown with the brackets: Pink 124 (42%), Red 87 (56%), Red 123 (61%), White 86 (63%), Gum 79 (96%). Black 210 was not included in this comparison since it is a synthetic rubber composition. Plotting of this effect *versus* percentage of rubber did not show a linear relationship but gave a smooth hyperbolic curve. Further investigation is required before an explanation of this phenomenon can be attempted.

In every case sorption in bisulfite solutions was markedly different from that in the other inorganic solutions. With four closures the sorption did not follow the log-log equation, but Gum 79, Black 210, and Red 123 were found to follow the equation $\log w = nt + k$, while Pink 124 with bisulfite solution followed the equation $w = nt + k$. If these equations were used to obtain extrapolation values such values would be enormous ($10^{12} - 10^{16}$ Gm.) making them seem unreasonable for use in comparisons. It is doubtful, therefore, that these equations are followed over more than a short period of time.

Bisulfite, instead of decreasing sorption of water, greatly increased it. This was found to be due partly to an increase in weight of the closure caused by bisulfite itself actually penetrating the closure and being weighed as water. This was proved by drying and weighing the closures at the end of the sorption experiments. This gain due to weight of salt within the closure accounted for only ten to twenty per cent of the total increase in weight, however, showing that there was a real increase in sorption of water also. It is believed that the bisulfite ion, or some derivative of it, is thus dissolved in the rubber and that it confers upon the rubber composition an increase in hydrophyllic character.

This penetration of rubber itself by an inorganic compound is the first instance noted in this or previous studies, and it is surprising, since inorganic compounds generally are not expected to be soluble in rubber. It appears to be peculiar to the bisulfite ion since both sodium and potassium bisulfites exhibited this effect but the corresponding thiosulfates did not penetrate the rubber and had only the expected vapor pressure effects common to the other inorganic compounds. A knowledge of chemical or physical reactions occurring in this process would perhaps give an insight into the reactions occurring between rubber and more complex molecules, and the need for further study is indicated.

The sorption tests were carried out in such a manner as to avoid accumulation of any material which might be leached from the rubber into the solutions. It was felt that the same method modified so as to allow accumulation of leached materials might be used to detect leaching. Any material leached from the stoppers, if soluble, would remain in the solution and thus lower the relative vapor pressure and decrease the sorption rate. To test this method, the sorption test was performed with the six closure types in the same manner as before but without changing the water during the test. So as to have values which were sure to be unaffected by leaching, the sorption test for comparison was run in water vapor instead of immersing the closures. The difference in the values obtained was expected to be indicative of amount of leaching.

A second method was also tested to detect leaching: Ten closures of each type were cut into small pieces and placed in 20-ml. glass ampuls. Ten milliliters of water were added, and the ampuls were sealed and autoclaved at 15 lb. pressure for one hour. After cooling, the ampuls were broken and the water decanted into five-dram vials. Water sorption by new closures of the same type was then determined from these autoclaved liquids without changing the liquids. A comparison of this sorption with that obtained with water vapor was also expected to be a measure of leaching.

Results of the leaching tests are shown in Table VI. Leaching would be indicated by a lower sorption as compared with that from water vapor. As seen in Table VI, there is a lower sorption in the

case of Gum 79 and Red 123 measured by both methods. The other closures show either no difference or a change in the opposite direction. When one considers the possibility that organic compounds may be leached from the rubber and that these might be rubber soluble and thus may be again absorbed by the fresh closure, it is not surprising that occasionally a higher rather than a lower sorption might be obtained in a test of this sort. Also, it is possible that materials leached may have very small effects on vapor pressure and that the test would thus be insensitive. These methods would, therefore, seem to have little promise as a general procedure.

TABLE VI.—RESULTS OF LEACHING TESTS
One-Year Extrapolation Values—Gm. × 10^4

Treatment	Gum 79	Pink 124	Red 123	Black 210	White 86	Red 87
Water vapor	443	445	508	187	508	334
Water—Method I	283	495	356	275	318	337
Autoclave liq.—Method II	290	647	374	278	566	345

SUMMARY AND CONCLUSIONS

1. Twenty inorganic salts were tested for their effect on sorption of water by a representative group of rubber closures. Comparisons of the effects of the ions were made by analyses of variance, using values extrapolated from the sorption curve.

2. Results showed that, with the exception of bisulfite ion, the effects of inorganic ions on the sorption by rubber closures could be explained entirely on the basis of their lowering of the vapor pressure of water. Comparisons of cations and anions gave the effects which would be expected on this basis; i. e., divalent cations depressed sorption to a greater extent than monovalent, and the phosphate anion, greater than the other anions tested.

3. The different rubber compositions were shown to be affected to different degrees in their sorption of water by inorganic salts, the order of effect being the same, except for one type containing synthetic rubber, with all the ions tested. The greater the rubber content of the composition the less the effect of the salts.

4. Bisulfite was found to give high sorption values, greatly increased over that of water, rather than depressing sorption as would be expected from vapor pressure considerations. This was shown to be due to penetration of rubber by bisulfite or some derivative of it, which changed the sorption characteristics of the rubber.

5. Two attempts at using sorption measurements as a means of detecting leaching met with only partial success.

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Notes

A Note on a Dehydration Product of *o*-Thymotic Acid*

By EDMUND GRANATEK and PAUL JANNKE

IN AN EARLIER REPORT (1), one of the dehydration products of *o*-thymotic acid was declared to be a dithymotide, m. p. 174°, mol. wt. 366 (Rast). Baker, *et al.* (2), has previously described the compound produced by the same method as a trithymotide, m. p. 172°, mol. wt. ebullioscopically 522 (benzene), 538 (chloroform) and 515 (carbon tetrachloride); theoretical 528. They described its physical and optical nature on the basis of their experience with other high-membered ring compounds. The optical properties mentioned were extended by Powell (3) in his "Thoughts on Optical Activity."

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In personal communication, Dr. Baker stated that our observations on the compound in question were in error, with difficulty arising from the fact that the compound was isolated with solvent of crystallization, thus giving a low molecular weight which was further complicated by a method they found to be unreliable for these kinds of compounds. He also supplied a sample of the trithymotide-ethanol complex.

When our samples of the compound in question were recrystallized in accordance with Dr. Baker's suggestion, namely from ethanol, dioxane, and petroleum ether, in order, the compound melting at 174° was obtained. When examined under magnification, the crystals were found to effloresce strongly at 160°, showing that solvent of crystallization was indeed present. A sample of the crystals was heated in a drying pistol over boiling cyclohexane (160°), at 6 mm. for nine hours. The solvent-free material showed the faintest tendency to effloresce at 215° and the sample melted sharply at 218°. The molecular weight of this compound was found to be 522 when determined ebullioscopically in benzene.

The behavior of the compound coincides with that described by Baker, *et al.*, for the trithymotide, and a parallel study on their trithymotide-ethanol complex shows the parent compounds to be identical. Therefore, our initial error in reporting on this compound is admitted and it is recognized that the compound is the trithymotide described originally by Baker, *et al.*

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A Note on Thioacetamide in Pharmaceutical Testing*

By ANTHONY J. MONTE BOVI

Thioacetamide was found to be a satisfactory replacement for hydrogen sulfide in certain official pharmaceutical tests used to: (*a*) establish the identity of mercury, bismuth, zinc, and manganese salts, and (*b*) detect the presence of such impurities as mercury in Merbromin and Nitromersol, mercuric iodide in yellow mercurous iodide, copper or zinc in methylene blue, iron in zinc oxide, and heavy metals in sodium chloride and in potassium nitrate.

FOR MORE THAN a century, hydrogen sulfide has been used routinely in both qualitative and quantitative chemical analyses. Its toxicity and nauseating odor have caused many to search for a satisfactory replacement capable of being freely used without discomfort. At one time or another, such substances as the thioacetates, thiocarbonates, thioglycollates, thiosalicylates, thiosulfates, and thioureas were suggested as possible replacements. But unfortunately, such substances have, at best, only a limited application in analytical chemistry.

The thioamides, although known for many years, were first shown to be capable of yielding hydrogen sulfide in analytical procedures by Vavilov (1). In 1949, Barber and Grzeskowick (2) found that thioacetamide not only was a satisfactory replacement but actually had many advantages over hydrogen sulfide when used in qualitative chemical analyses. Others (3–7) soon found thioacetamide a satisfactory replacement for hydrogen sulfide as a precipitating agent in many different types of chemical analyses.

Properties and Uses of Thioacetamide.—Thioacetamide is a fine, white powder, readily soluble in both alcohol and water. It is usually employed as a 2 to 10% aqueous solution. Such solutions are stable at room temperatures (20–30°), although a slight turbidity may develop in solutions which have been stored for long periods of time. Even so, filtration and subsequent use of the filtrate does not seem to appreciably affect the precipitating power of the solution.

At temperatures exceeding 80°, thioacetamide begins to hydrolyze, the rate of hydrolysis depending on the temperature and *p*H of the solution. Both acids and bases seem to catalyze this hydrolysis, the reactions taking place are believed to be as follows: In acids: $CH_3CSNH_2 + 2H_2O \rightarrow H_2S + CH_3COONH_4$; in bases: $CH_3CSNH_2 + 3OH^- \rightarrow CH_3COO^- + NH_3 + S^{--}$

Thioacetamide generates hydrogen sulfide where the reaction is to take place. The hydrogen sulfide so evolved is immediately reacted upon, thus allowing no escape of the gas from the reaction vessel. Hence, the most disagreeable aspects of hydrogen sulfide, its toxicity, and nauseating odor, are under control.

In general, thioacetamide can be used to precipitate certain metallic ions which yield insoluble sulfides. It is necessary, of course, that in such cases the same conditions of concentration and *p*H as prevail when using hydrogen sulfide be maintained. Swift and Butler (8) have shown that thioacetamide hydrolyzes with varying degrees, depending greatly on temperature and *p*H. Hence, the indiscriminate use of this reagent as a direct replacement for hydrogen sulfide is not justified. This is particularly pertinent in those analytical procedures using techniques which would cause a secondary hydrolysis of the acetamide produced in the reaction to take place. The ammonia, produced in this hydrolysis would make uncertain the *p*H concentration of the solution. However, under

* Received April 13, 1956, from St. John's University, College of Pharmacy, Brooklyn, N. Y.

the conditions stated in our experimental procedure, the secondary hydrolysis of acetamide does not proceed to such an extent as to cause difficulty and adjustment of the *p*H.

As used in this study, thioacetamide can be used with only slight modification of the procedures requiring the use of hydrogen sulfide. There is no need for extensive development, or experimentation with radically new procedures since, as a rule, in the experimental examples given, the methods suitable with hydrogen sulfide will yield, with slight modification, to similar treatment with thioacetamide.

Experimental Procedure.—Although Iwanov (5) used a freshly prepared and purified thioacetamide for the detection of heavy metals in certain pharmaceutical preparations as early as 1935, it was decided to reinvestigate the possibility of using commercially available thioacetamide in those pharmaceutical tests which now call for the use of hydrogen sulfide.

In general, the pharmaceutical tests requiring the use of some form of hydrogen sulfide fall into one of two categories; tests to (*a*) establish the identity of a substance, or (*b*) detect the presence of certain impurities in the substance.

Tests were conducted using the following procedures, unless otherwise specified:

A solution of the substance to be tested was prepared according to the directions of the compendia. A slight excess of a 5% (w/v) thioacetamide solution was then added, and the mixture heated on a water bath for five minutes.

Identification Tests

Mercury in Mercury Bichloride U. S. P. (9).—A black precipitate, insoluble in dilute nitric acid was produced.

Bismuth in Bismuth Subcarbonate U. S. P.—When dissolved in a slight excess of nitric or hydrochloric acid, a white precipitate formed upon dilution with water. Subsequent treatment with the thioacetamide solution caused the formation of a brown precipitate, soluble in dilute nitric acid.

Zinc in Zinc Oxide U. S. P.—In the presence of sodium acetate, a white precipitate was formed, which was insoluble in acetic acid but soluble in dilute hydrochloric acid. Also, a white precipitate formed with a neutral zinc solution when the thioacetamide was first rendered slightly alkaline.

Manganese in Manganese Glycerophosphate N. F. (10).—A salmon-colored precipitate formed when the thioacetamide solution, first rendered alkaline with ammonium hydroxide T. S., was added and the mixture heated for ten minutes. The precipitate dissolved in acetic acid.

In all the above-mentioned tests, the official procedure was carried out simultaneously as a control. The thioacetamide solution produced similar results in all instances.

Tests for Impurities

These tests were carried out on both pure and contaminated samples. An amount of the impurity sought was added to the sample until a well-defined positive test was obtained. This amount was then progressively decreased until the official test no longer detected the impurity. This second amount was then increased until a quantity, capable of being easily detected, was found. With the exception of the heavy metals limit tests for sodium chloride and potassium nitrate, where the degree of contamination can be quantitatively controlled by the standard lead solution used in the test, no attempt was made to determine the exact concentration of impurity which could be detected. This phase of the problem is being considered as part of a separate study on the sensitivity of thioacetamide when used in such tests. At present, it appears that thioacetamide and hydrogen sulfide are about equal in the ability to detect the ions mentioned. Our experience has shown that in those dilutions where hydrogen sulfide failed to detect an ion, thioacetamide did likewise. In those dilutions where hydrogen sulfide was able to detect the ion, thioacetamide did likewise. In all instances, no reaction was observed when the pure samples were treated with the thioacetamide solution. The contaminated samples reacted as follows:

Mercury in Merbromin N. F. and in Nitromersol N. F.—A distinct darkening in color was produced.

Mercuric Iodide in Yellow Mercurous Iodide N. F.—The color of the sample was darker than that of a control.

Copper or Zinc in Methylene Blue N. F.—A turbidity was produced in the sample contaminated with zinc. The color of the sample contaminated with copper was darker than that of the control.

Iron in Zinc Oxide U. S. P.—A distinct darkening of the precipitate was produced, when the thioacetamide, first rendered alkaline with sodium hydroxide T. S., was added.

U. S. P. Heavy Metals Limit test for Sodium Chloride U. S. P. and Potassium Nitrate N. F. In both instances, the color of solution *B* (contaminated sample) was darker than that of solution *A* (control).

SUMMARY

Despite the fact that thioacetamide has been known for many years, its potential as a replacement for hydrogen sulfide in pharmaceutical analysis is only beginning to be realized. Many applications in pharmaceutical testing can be found for this substance. There is need, however, for more fundamental studies, especially on a quantitative basis, to establish (*a*) the sensitivity of thioacetamide, and (*b*) the mechanisms of reaction, before it can be unequivocally adopted as a direct replacement for hydrogen sulfide in all pharmaceutical testing. Until then, the indiscriminate use of thioacetamide as a replacement for hydrogen sulfide is not justified.

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An Improved Method for Drop Recording*

By CLINTON B. NASH

A simple, trouble-free method for counting and recording drops of fluids in biological experiments is described.

THERE HAVE BEEN a number of ingenious methods reported for counting and recording drops of fluid (1–8). While most of these perform satisfactorily under certain restricted conditions, all of them have some defects. A common feature of many methods involving fluids containing electrolytes is the completion of a circuit by a drop falling between two contact points and it is this portion of the equipment that is most prone to cause difficulty. Drops either hang on the electrodes and maintain a contact or else they drop without completing the circuit.

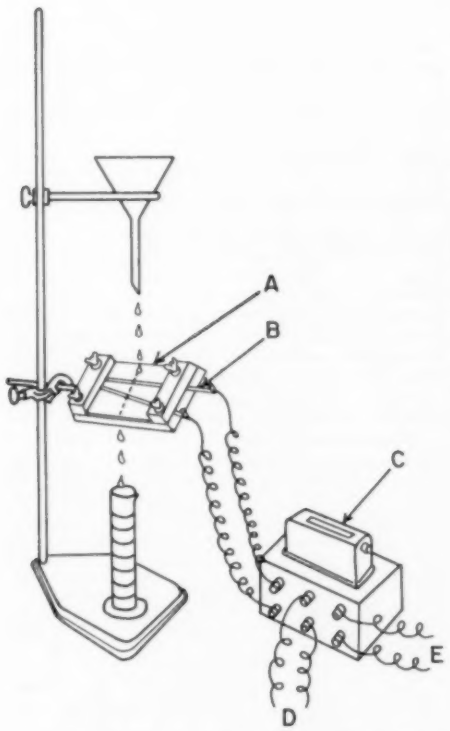

Fig. 1.—Sketch of dropping contacts and electronic counter. (*A*) lucite board with thin layer of paraffin. (*B*) metal contact strips. (*C*) electronic counter. (*D*) leads to signal magnet. (*E*) leads to 3-volt battery.

The drop contacts illustrated in Fig. 1 eliminate these difficulties. The contacts are thin flat strips of copper (or other suitable metal), 3 mm. wide and 1 mm. thick, held in place by a lucite bar and thumb bolts at each end of a lucite base measuring 14 by 8.5 cm. Temporary side boards are held in place while melted paraffin is poured over the surface of the contact board until the electrodes are completely covered. After hardening, the paraffin is carefully scraped down to a level with the copper contacts. The metal strips are angled slightly so that at one end they are 12 mm. apart and at the other, 24 mm. distant. The divergence of the contacts permits adjustment of electrode distance by simply moving the contact board to cause the drops to fall nearer one end or the other. Tilting the board toward the vertical plane increases the speed of the drop and "stretches" it across the contacts, as does increasing the dropping distance. By use of these three adjustments the contacts may be fitted to the particular fluid being counted and adjustments can be rapidly made during an experiment. The tendency of very viscous fluids to

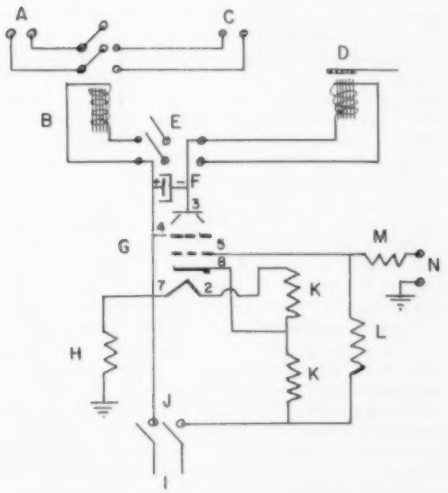

Fig. 2.—Wiring diagram for electronic counter. (*A*) 3-volt battery. (*B*) Advance relay #1504, 1600 ohm coil. (*C*) Signal magnet. (*D*) Veeder-Root counter. (*E*) Double pole double throw switch. (*F*) 4 Microfarad capacitor, 150 volts. (*G*) RCA tube 50L6. (*H*) 330 K to 470 K ohms, 1 watt resistor. (*I*) Line. (*J*) Double pole single throw switch. (*K*) 250 Ohm, 10-watt resistor. (*L*) 2.2 Megohm to 10 megohm, 1 watt resistor. (*M*) 36 K to 270 K, 1 watt resistor. (*N*) Dropper electrodes.

* Received June 11, 1956. From the Department of Pharmacology, Mead Johnson Research Laboratory, Evansville, Ind.

maintain a bridge across the contacts is corrected by wiping a few drops of Dow-Corning fluid 200 over the face of the contact board or by having the drops displace a saline solution. The drops may be collected in a graduate for volume measurements if desired.

Any one of several electronic counters may be used in conjunction with the above electrodes. The one in use in this laboratory was furnished through the courtesy of Dr. R. V. Brown (9) and the circuit is shown in Fig. 2. This increases the sensitivity of the system so that drops having a very low electrolyte content can be counted satisfactorily. A Veeder-Root counter, 110 volts, 8 watts, may be included if desired.

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Letters to the Editor

The Ion-Exchange Chromatographic Determination of Antihistamines

Sir:

We wish to point out that the procedure described for the determination of antihistamines in a recent article (1) is not new. This method, applied to the determination of antihistaminics and some dosage forms of them, was previously described by us (2) and an abstract of our article was published in *Chemical Abstracts* (3). Our original article included a description and a report of the results obtained in the determination of ethanolamine- and ethylenediamine-type antihistaminics. The procedure was also applied in the assay of Probedryl "Probel" [2-(benzohydryloxy)-N,N-dimethylethylamine hydrochloride] and Phenergan "Specia" [10-(2-dimethylamino-1-propyl)-phenothiazine hydrochloride]. The article emphasized that the method could also be used for the analyses of other antihistaminics since it had been employed successfully for the assay of the most important chemical groups represented in this category. Our article was cited in a review (4) of the application of ion-exchangers in pharmacy and is commonly recognized (5, 6). It is certainly very surprising that Blaug and Zopf (1), referring in their work to all sorts of determinations of antihistaminics, completely ignore our work, which from the point of view of using ion-exchangers, is quite fundamental. Moreover, these authors describe as new, an analytical method, which previously had been developed and reported (2) by us.

In consideration of the above information, we believe we are entitled to a priority status for the utilization of a strongly basic ion-exchanger (Amberlite IRA 400) in the analyses of antihistaminics and their dosage forms.

Antonin Jindra
Otakar Motl
Biochemical Institute, Charles University
Prague, Czechoslovakia

Sir:

It has been called to our attention that an article dealing with an ion-exchange method for the determination of antihistamines appeared in *Československ. farm.* prior to the publication of our article in THIS JOURNAL. Although the method used in our study is similar to that used by Jindra and Motl, it should be pointed out that the method is not applicable to all dosage forms of antihistamines as claimed by Jindra. The presence of alkali and ammonium salts interferes markedly in the analysis of galenicals containing an antihistamine. Strongly basic anion exchangers not only liberate the antihistamine base, but also convert alkali and ammonium salts quantitatively into the corresponding bases. Amberlite IR-4B, acting as a weak base, can be used whenever the pharmaceutical preparation to be analyzed contains these salts. Furthermore, preparations containing ephedrine cannot be assayed by Jindra's method. A method for determining an antihistamine in an ephedrine-containing preparation is presented in our study.

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Book Notices

Textbook of Organic Medicinal and Pharmaceutical Chemistry. 3rd ed. By CHARLES O. WILSON and OLE GISVOLD. J. B. Lippincott Company, Pa., 1956. xvi + 823 pp. 17.5 x 26 cm. Price $11.

The third edition of this text brings it up-to-date with the U. S. P. XV and N. F. X. Various sections of the text have been revised by the following authors: T. C. Daniels, Robert F. Doerge, Ole Gisvold, W. D. Kumler, E. V. Lynn, T. O. Soine, Abraham Taub, G. L. Webster, and Charles O. Wilson. The text is introduced by chapters on: physicochemical properties in relation to biologic action, and metabolic changes of drugs and related organic compounds. Each of the general subdivisions of therapeutic agents, diagnostic aids, and pharmaceutical aids is introduced with a discussion of the group of compounds. This is followed by a descriptive treatment of individual items. Official status and occurrence in dosage forms are indicated. Many drugs are grouped according to their pharmacologic actions. This textbook should be useful in courses on medicinal and pharmaceutical chemicals and should be included in all pharmacy libraries for its value as a reference volume. Text references are given at the end of each chapter and a subject index is appended.

Blakiston's New Gould Medical Dictionary. 2nd ed. By NORMAND L. HOERR and ARTHUR OSOL. McGraw-Hill Book Company, Inc., New York, 1956. xxvi + 1463 pp. 17.5 x 25.5 cm. Price $11.50.

This new edition makes a good medical dictionary even better. Modernized usage and spellings of terms are included, and about 12,000 items have been added. Drugs which are still in different stages of animal and clinical investigation are defined in this up-to-the-minute effort. The full and dependable coverage of the drug field is obviously attributable to co-editor Arthur Osol. He and Normand L. Hoerr, with two other members of the editorial board and eighty-eight contributors, have prepared a very useful volume. Dr. Osol's knowledge in the field of nucleonics is evident in the full treatment of the new terminology relating to subatomic phenomena. No library is complete without a good medical dictionary, and this one is excellent.

Currents in Biochemical Research 1956. By DAVID E. GREEN. Interscience Publishers, Inc., New York, 1956. xvi + 697 pp. 16 x 28.5 cm. Price $10.

This book is a compilation of the efforts of twenty-seven selected contributors to prepare reviews of their special fields of biochemical research covering the past ten years and indicating present and future trends of study. The scope of the coverage is indicated by the titles: chemistry and viral growth; photosynthesis; bacterial fermentations; some aspects of vitamin and growth factor research; the significance of induced enzyme formation; certain problems in the biochemical study of disease; the hormones, their present significance, their future; problems of cellular biochemistry; enzymes as reagents; attempts at the formulation of some basic biochemical questions; enzyme complexes and complex enzymes; relations between prosthetic groups, coenzymes and enzymes; enzyme-substrate compounds and electron transfer; on the nature of hemoprotein reactions; aspects of protein structure; the structure of insulin; a new concept of ribonucleic acids; chemical structure as a guide to the study of biochemical syntheses; the role of nucleotides and coenzymes in enzymatic processes; the biosynthesis of porphyrins, the succinate-glycine cycle; problems in the study of multiple enzyme systems; enzyme kinetics; the interconversion of sugars in nature; a theory of the primary event in muscle action; trends in the biochemistry of nerve activity; blood; some functional considerations; and an integrated concept of carcinogenesis.

The great strides in the advancement of biochemical research and knowledge are attributed to a large degree to the developments in methodology and to the new tools for analytical studies, such as chromatography and spectrophotometry. The new fields for study include the biochemical description of muscular contraction, nerve conduction, glomerular filtration, and secretion and membrane phenomena. References are given at the end of each discussion. The format and printing of the book are very good. A general index would be helpful to those who would use this compilation as a reference text. Undergraduates taking biochemistry, as well as graduate students and those active in applied research, will want this book available to them.

Advances in Enzymology. Vol. 17. Edited by F. F. NORD. Interscience Publishers, Inc., New York, 1956. v + 556 pp. 16 x 24 cm. Price $11.

This volume is a compilation of chapters contributed by different authors on the following subjects: enzyme kinetics; the respiratory chain and oxidative phosphorylation; solubilization, migration, and utilization of insoluble matter in nature; enzymatic phosphate transfer; the formation of oligosaccharides by enzymic transglycosylation; nature and function of metalloflavoproteins; chemistry and biochemistry of xanthine oxidase; some controversial aspects of the mammalian cytochromes; metabolic aspects of chemical genetics; and ribonucleic acids and virus multiplication. References are given at the end of each chapter. Author and subject indexes for this volume and cumulative author and subject indexes for volumes 1–17 are appended. The book is a useful addition to biochemical libraries.

Acridines. The Chemistry of Heterocyclic Compounds, Vol. IX. By R. M. ACHESON. Interscience Publishers, Inc., New York. xii + 409 pp. 15.5 x 23.5 cm. Price $12.50.

This is one of a series of monographs on the various branches of heterocyclic chemistry. The material on acridines is introduced by a discussion on nomenclature and numbering system. This is followed by detailed information on methods of preparation, properties, and reactions under the chapter headings: acridines, aminoacridines, acridones, acridine alkaloids, acridinium salts and reduced acridines, biacridines, visible and ulraviolet absorption spectra of acridine and its derivatives (by L. E. Orgel), benzacridines, condensed acridines and acridine dyes, and biologically active acridines. The book should be an excellent reference volume. The text contains many references, 400 references are included in the 70-page chapter on acridines. Tabulated data, spectral curves, and structural formulas are used freely. An index is appended.

Textbook of Pharmacognosy. 3rd ed. By T. E. WALLIS. J. & A. Churchill Ltd., London, 1955. (Distributed by Little, Brown, and Company, Boston, Mass.) xi + 578 pp. Illustrated. 15 x 22.4 cm. Price $7.50.

This standard English textbook follows the plan of earlier editions in using a morphological classification scheme, such as previously was so popular in U. S. texts. [For a general discussion of the subdivision of subject matter, see review of Edition 1 in THIS JOURNAL, **35,** 320(1946).]

Wallis' text presents a good example of careful workmanship with relatively few printers' errors in contrast with the rather high frequency of such that mar comparable American texts. An unusual feature of this, as of other British texts in this field, is the inclusion of mineral drugs (chalk, talc, asbestos, kaolin) which of course as crude natural materials rightfully belong here.

As in previous editions, the drawings (largely original) are excellent primarily because simple diagrams are used rather than detail sketches or photographs which often prove almost useless. (Thus, Fig. 167 shows line drawings of Indian valerian, with diagrams of rhizome, stolon, and root cross-sections.) The more detailed drawings are often borrowed from other works.

An obvious criticism of the book for American usage is the citation of British compendia and an understandable disregard for both the U. S. P. and the N. F. Consequently a number of drugs taken up are of little or no interest to the American student. On the other hand, and this is a more serious defect, some drugs and related materials which are moderately to very important in the United States are not included.

Although the book is not as voluminous as some in the American field, it has been written in so succinct a manner that not only is the coverage adequate for class use, but the work also provides much useful reference data not always available in other works of like category. An excellent feature new to this edition is an appended table of reference citations.

Despite the effective deletion of obsolete material, there is a considerable increase in number of items and pages of text, manifesting again the continued development of the subject field.

BOOKS RECEIVED

Perspectives in Organic Chemistry. Edited by SIR ALEXANDER TODD. Interscience Publishers, Inc., New York, 1956. x + 527 pp. 15 x 23.5 cm. Price $7.50.

An Introduction to Modern Organic Analysis. By SIDNEY SIGGIA and HANS J. STOLTEN. Interscience Publishers, Inc., New York, 1956. vii + 250 pp. 15.5 x 24 cm. Price $4.50.

Die äusseren Heilmittel 1950–1955. By HERMANN v. CZETSCH-LINDENWALD and FRIEDRICH SCHMIDT-LABAUME. Springer-Verlag, Berlin, 1956. vii + 149 pp. 15.5 x 23.5 cm. Price DM 19.60.

The Chemistry and Technology of Waxes. New 2nd ed. By ALBIN H. WARTH. Reinhold Publishing Company, New York, 1956. vii + 940 pp. 16 x 23.5 cm. Price $18.

The Condensed Chemical Dictionary. 5th ed. By ARTHUR and ELIZABETH ROSE. Reinhold Publishing Corporation, New York, 1956. xix + 1200 pp. 16 x 23 cm. Price $12.50.

Sleep. By MARIE CARMICHAEL STOPES. Philosophical Libary, Inc., New York, 1956. 154 pp. 14 x 22 cm. Price $3.

1957 Pharmacy State Board Questions and Answers. By RALPH J. MILL, College of Pharmacy, Wayne State University, Detroit, Michigan. Clark & Wilson Co., Detroit, Michigan, 1956. 21 x 28 cm.

Chemistry of Carbon Compounds. Vol. IIIB. (Aromatic Compounds.) Edited by E. H. RODD. D. Van Nostrand Company, Inc., New York, 1956. xviii + 1,669 pp. 15 x 23 cm. Price $25.

Psychiatric Research Reports. Papers presented at the Mid-Atlantic Regional Research Conference. American Psychiatric Association, Washington, D. C., 1956. 153 pp. 15 x 22.5 cm Price $2.

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